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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

#17
8-21-03

In re Application of McBride and Griffiths
U.S. Serial No.: 09/676,783
Filed: October 2, 2000
Attorney Docket No.: 018733/0997
For: RADIOMETAL-BINDING PEPTIDE ANALOGUES

BRIEF ON APPEAL

Appeal from Group 1639

FOLEY & LARDNER
Suite 500
3000 K Street, N.W.
Washington, DC 20007-5109

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Appellants hereby appeal the January 13, 2003 rejection of the above-identified application to the Board of Patent Appeals and Interferences.

I. REAL PARTY IN INTEREST

Immunomedics, Inc., Morris Plains, NJ, owns the entire right, title and interest in the present patent application, as evidenced by an assignment recorded on 01/20/1998, at Reel 8927, Frame 0059. Immunomedics is, therefore, the real party in interest.

II. RELATED APPEALS AND INTERFERENCES

Appellants are aware of no other appeals or interferences pertaining to the instant invention.

III. STATUS OF CLAIMS

Claims 24 - 43 are pending. All claims ultimately depend from claim 24. A copy of the claims, as amended thus far, is presented in APPENDIX I.

IV. STANDING TO APPEAL CLAIMS 24 – 40, 42 and 43

Claim 24, the rejected base claim, was first rejected in paper number 9, on July 31 2002. Claim 24 was again rejected on January 13, 2003, in paper number 11. Since this claim has been twice rejected, Appellants submit that appeal of the present non-final rejection is proper in accord with 37 C.F.R. § 1.191(a).

V. SUMMARY OF THE INVENTION

The invention relates to derivatives of biologically useful cyclic and acyclic peptides in which one or more amino acid side chains or a segment attached to the peptide chain contain chelating moieties that can tightly bind metal ions, including radionuclides. The labeled peptides carry the metal to specific *in vivo* targets such as receptors and antigens, and are useful for radiodiagnostic imaging, therapy and radiotherapy. The peptides can bind radionuclides while retaining the ability to specifically bind to the peptide receptor. The

radiolabeled peptides can then be used to image or treat a tumor, an infectious lesion, a myocardial infarction, a clot, an atherosclerotic plaque, or a normal organ or tissue.[1]

Radiolabeled peptides such as those claimed in this application are useful in the diagnosis and therapy of a variety of human disease states that are characterized by overexpression of peptide hormone receptors. Thus, for example, it has been shown that radiolabeled analogues of LHRH (luteinizing hormone releasing hormone) and somatostatin selectively bind to hormone-sensitive tumors characterized by cell-surface overexpression of LHRH hormone receptors. Similarly, peptide hormone analogues such as ^{123}I-vasoactive intestinal peptide (VIP), ^{99m}Tc-P829, ^{111}In-DTPA Octreotide and ^{111}In-bisMSH-DTPA have been used to image human tumors that over express VIP, somatostatin, and melanocyte stimulating hormone (MSH) receptors. *See* Virgolini *et al.*, *Engl. J. Med. 169*:1116 (1994); Virgolini *et al.*, *J. Nucl. Med. 36*:1732, (1995); Pearson *et al.*, *J. Med. Chem. 39*:1361, (1996); Krenning *et al.*, *J. Nucl. Med. 33*:652 (1992); and Wraight *et al.*, *Brit. J. Radiol. 65*:112 (1992).[2]

VI. ISSUE ON APPEAL

The sole issue on appeal is whether claims 24 - 40, 42 and 43 comply with 35 U.S.C. § 112, first paragraph.

Claims 24 - 40, 42 and 43 stand rejected in a January 13, 2003 Office Action on the grounds that the aforementioned claims contain subject matter which was not described in the Specification in such a way as to reasonably convey to one skilled in the relevant art, that the inventors, at the time the application was filed, had possession of the claimed invention. The PTO alleges that at the time appellant's application was filed, the extent to which the claimed radiolabeled cyclic peptides have been used is only in the diagnosis of specific tumors. The PTO has taken the position that treatment of even a specific tumor with the claimed radiolabeled cyclic peptides, if anything, simply looks promising.

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[1] See page 1, lines 6 – 14 of the Specification.
[2] See page 1, lines 15 – 34 of the Specification.

VII. GROUPING OF THE CLAIMS

The claims all stand or fall together.

VIII. SUMMARY OF THE ARGUMENT

The PTO erred when it held that claims contain subject matter which was not described in the Specification in such a way as to reasonably convey to one skilled in the relevant art, that the inventors, at the time the application was filed, had possession of the claimed invention.

Contrary to the PTO's position, Appellants were in possession of the claimed invention: a method of treating a tumor using the claimed radiolabeled peptides. First, the Specification describes radiolabeling and *in vitro* experiments. Second, at the time the application was filed, the level of skill in the art to which the invention pertains was such that it is not necessary for Appellants to include much more detail in the Specification, in addition to the radiolabeling and *in vitro* experiments described therein, to demonstrate that they were in possession of a method for treating a tumor using the claimed radiolabeled peptides. Indeed, at the time the application was filed, it was known that many peptides closely related to those claimed in claim 24 could be used in radionuclide therapy to treat tumors. The Specification, therefore, is enabling for the treatment of tumors with the claimed radiolabeled peptides. Thus, claim 24, and the claims which depend upon it, should not have been rejected under 35 U.S.C. § 112, first paragraph for allegedly lacking enablement.

IX. ARGUMENT

In the Office Action dated July 31, 2002, the PTO asserts that the claims contain subject matter which was not described in the Specification in such a way as to reasonably convey to one skilled in the relevant art, that the inventors, at the time the application was filed, had possession of the claimed invention.

In the response to the July 31, 2002 Office Action,[3] Appellants rebutted the PTO's assertions by relying on MPEP Section 2163 which provides, in relevant part, that the analysis of whether the Specification complies with the written description requirement calls for the PTO to compare the scope of the claim with the scope of the description to determine whether applicant has demonstrated possession of the claimed invention. Such a review is conducted from the standpoint of one of skill in the art at the time the application was filed (see, e.g., *Wang Labs. v. Toshiba Corp.*, 993 F.2d 858, 865, 26 USPQ2d 1767, 1774 (Fed. Cir. 1993)) and should include a determination of the field of the invention and the level of skill and knowledge in the art. The guidelines in Section 2163 themselves provide that "***[g]enerally, there is an inverse correlation between the level of skill and knowledge in the art and the specificity of disclosure necessary to satisfy the written description requirement.***" Information which is well known in the art need not be described in detail in the Specification. *See*, e.g., *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 802 F.2d 1367, 1379-80, 231 USPQ 81, 90 (Fed. Cir. 1986).

In support of Appellants' position, Appellants submitted journal articles that illustrated the level of skill in the art to which the invention pertains. These articles demonstrated that, at the time the application was filed, it was known that peptides related to those claimed in the present application where promising *in vitro* candidates for the treatment of certain tumors. Further, these articles demonstrated that the *in vitro* results could be extrapolated to the use of such peptides in radionuclide therapy to treat certain tumors *in vivo*.[4] In the response to the

[3] See page 6 of the Response filed October 31, 2002.

[4] The U.S. Court of Appeals for the Federal Circuit, in *Rey-Bellet* and *Kawai*, has implied that "a particular pharmacological activity identified with prior art compounds may have probative value as to the fact that the compound of the [invention] possesses this particular pharmacological activity where there is a structural similarity between the prior art compounds and the compound of the [invention]." *Cross v. Iizuka* 753 F.2d 1040, 1048 (Fed. Cir. 1985) citing *Rey-Bellet v. Engelhardt*, 493 F.2d 1380, 1385 (CCPA 1974); *Kawai v. Metlestics*, 480 F.2d 880, 890 (CCPA 1973). Although Appellants have provided journal articles which describe how peptides related to those claimed in the present application have been used for the treatment of certain tumors *in vivo*, Appellants offer that it is well settled that *in vitro* data is sufficient, in many cases, to satisfy the utility and the enablement requirement of 35 U.S.C. § 112, first paragraph. *See Cross v. Iizuka*, 753 F.2d 1040, 1050 (Fed. Cir. 1985) ("*in vitro* results with respect to the particular pharmacological activity are generally predictive of *in vivo* results, i.e., there is a reasonable correlation therebetween.").

January 13, 2003 Office Action, Appellants submitted additional articles that demonstrated that the extrapolation of *in vitro* results to *in vivo* radio therapeutic efficacy was indeed appropriate. These articles demonstrated that peptides related to those claimed in the present application can and have been used in radionuclide therapy to treat tumors in rat animal models and in human patients.

The journal articles which demonstrated *in vitro* and *in vivo* results using peptides related to those claimed in the present application are summarized below.

In vitro

de Jong *et al.*, *Cancer Research 58*: 437-441 (1998) (EXHIBIT A)

de Jong *et al.* demonstrated that ^{111}In-labeled somatostatin analogs showed high and specific binding *in vitro* to somatostatin receptors in mouse pituitary AtT20 tumor cell membranes. de Jong *et al.* also showed that all of the compounds that were evaluated, namely, [DTPA^0]octreotide, [DTPA^0,Tyr^3]octreotide, [DTPA^0,D-Tyr^1]octreotide, [DTPA^0,Tyr^3]octreotate, and [DOTA^0,Tyr^3]octreotide, showed specific internalization in rat pancreatic tumor cells. In addition, de Jong *et al.* showed that these results translated to *in vivo* models. For example, biodistribution studies showed that radioactivity in the octreotide-binding, receptor expressing tissues and tumor-to-blood ratios were significantly higher when [^{111}In-DTPA^0,Tyr^3]octreotide, [^{111}In-DTPA^0,Tyr^3]octreotate, and [^{111}In-DOTA^0,Tyr^3]octreotide were used than when [^{111}In-DTPA^0]octreotide was used. Finally, de Jong *et al.* characterize radiolabeled [DTPA^0,Tyr^3]octreotide, and especially [DTPA^0,Tyr^3]octreotate and their DOTA-coupled counterparts as "most promising for scintigraphy and radionuclide therapy of [somatostatin] receptor-positive tumors in humans.

Lewis *et al.*, *J. Med. Chem. 42*: 1341-1347 (1999) (EXHIBIT B)

In a study which illustrated the structure activity relationship of various somatostatin analogs related to those described by de Jong *et al.* (*supra*), Lewis *et al.* compared the *in vitro*

binding, *in vitro* tumor cell uptake, and *in vivo* distribution of [^{64}Cu-TETA,Tyr^3]octreotide and [^{64}Cu-TETA]octreotate with that of [^{64}Cu-TETA,Tyr^3]octreotate and [^{64}Cu-TETA]octreotide. Appellants note that they have used the same type of nomenclature used in the de Jong *et al.* to describe the peptides of Lewis *et al.* Lewis *et al.* demonstrated that, while all of these peptides displayed affinity for somatostatin receptors on CA20948 rat pancreatic tumor membranes, [^{64}Cu-TETA]octreotate and [^{64}Cu-TETA,Tyr^3]octreotate showed the highest affinity for the receptors. Biodistributions in CA20948 tumor-bearing rats showed receptor mediated uptake of the ^{64}Cu-labeled peptides in somatostatin-rich tissues, including the pituitary adrenals, pancreas, and tumor. Lewis *et al.* found that [^{64}Cu-TETA,Tyr^3]octreotate exhibited the highest tumor uptake of all of the peptides studied.

Lewis *et al. Clinical Cancer Research 5*: 3608-3616 (1999) (EXHIBIT C)

In this study, Lewis *et al.* mention a previous study which showed that [^{64}Cu-TETA]octreotide significantly exhibited the growth of somatostatin receptor-positive CA20948 rat pancreatic tumors in Lewis rats. Anderson *et al.*, *J. Nucl. Med. 39*: 1944-1951 (1998). In the current study, Lewis *et al.* found that a single dose of 15 mCi of [^{64}Cu-TETA,Tyr^3]octreotate was shown to be more effective in reducing tumor burden than the same dose of [^{64}Cu-TETA]octreotide. Lewis *et al.* also found that in multiple dose experiments, complete regression of tumors was observed for all rats treated with 3 x 20 mCi of [^{64}Cu-TETA,Tyr^3]octreotate; with no palpable tumors for approximately 10 days. Lewis *et al.* found that the mean survival time of the rats was nearly twice that of controls.

In vivo

Rat Animal Model

Bugaj *et al. Nucl. Med. Biol. 28*: 327-334 (2001) (EXHIBIT D)

Using animal tumor models, Bugaj *et al.* evaluated the radiotherapeutic efficacy of the radiolabeled somatostatin analog CMDTPA-Tyr^3-octreotate: a compound related to the

radiolabeled peptides claimed in the present invention. Bugaj *et al.* focused on the beta-emitting nuclide, ^{153}Sm, chelated to the somatostatin analog, CMDTPA-Tyr^3-octreotate. Bugaj *et al.* found that suppression of tumor growth rate was observed in all animals treated with ^{153}Sm-CMDTPA-Tyr^3-octreotate compared to untreated controls. Greater inhibition of tumor growth was observed in animals that received multiple doses.

On page 332, column 2, of the Bugaj *et al.* article, it is mentioned that "[a]dditional studies are necessary to determine whether the high pancreatic uptake observed in rats will also be found in humans." Bugaj goes on to say that "[r]esults with other octreotate derivatives in primates, where no apparent pancreas uptake is observed in scintigraphs, suggest that this will not be the case." Appellants note, and the skilled artisan will recognize, that tumors in locations other than the pancreas may be treated using the compound reported by Bugaj *et al*, notwithstanding Bugaj *et al.*'s comments vis-à-vis testing of the reported compounds in primates.

Human Studies: Beyond the Rat Animal Model

Paganelli *et al.*, *Cancer Biother. Radiopharm. 14*: 477 – 483 (1999) (EXHIBIT E)

When the instant application was filed, Paganelli *et al.* had already demonstrated that a compound related to the radiolabeled peptides claimed in the present invention, can be used to treat tumors in humans. Paganelli *et al.* reports the dosage, safety profile and therapeutic efficacy of ^{90}Y-labeled DOTA-[D-Phe^1-Tyr^3]-octreotide (DOTATOC) when patients with cancers expressing somatostatin receptors are treated with this compound. Paganelli *et al.* also showed that out of 5 patients that were treated, complete and partial tumor mass reduction was measured in 25% of patients, along with 55% showing stable disease and 20% showing progressive disease.

In a 2001 journal article, Paganelli *et al.* reported the results from treatment of 30 patients with DOTATOC. Paganelli *et al.*, *Eur. J. Nucl. Med. 28*: 426 – 434 (2001). Paganelli *et al.* demonstrated that complete or partial tumor mass reduction occurred in 23% of

patients; 64% had stable and 13% progressive disease. Both of the reports by Paganelli *et al.* are congruent with the notion that compounds such as those claimed in the present invention can be used to treat tumors in humans.

Kwekkeboom *et al.*, *Eur. J. Nucl. Med. 28*: 1319-1325 (2001) (EXHIBIT F)

Kwekkeboom *et al.* recognized and demonstrated that ^{177}Lu- and ^{111}In-labeled somatostatin analogs were effective in treating tumors in animal models. For example, when the somatostatin analog [DOTA^0,Tyr^3]octreotate, a compound related to the compound used by Paganelli *et al.*(*supra*) and to the compounds claimed in the present invention, was labeled with the beta- and gamma-emitting radionuclide ^{177}Lu, it had a favorable impact on tumor regression and animal survival in a rat model. Because of these advantages Kwekkeboom decided to compare [^{177}Lu-DOTA^0,Tyr^3]octreotate with [^{111}In-DTPA^0]octreotide in six *human* patients with somatostatin receptor-positive tumors. From their comparative experiments, Kwekkeboom *et al.* concluded that [^{177}Lu-DOTA^0,Tyr^3]octreotate demonstrated higher absorbed doses in most tumors, with about equal doses to potentially dose-limiting organs.

In addition to their own findings, Kwekkeboom *et al.* report a study by Otte *et al.* that showed that five human patients suffering from neuroendocrine tumors were treated successfully with [^{90}Y-DOTA^0-Tyr^3]octreotide. The Kwekkeboom *et al.* article also cites results of a study by Valkema *et al.* using [^{90}Y-DOTA^0-Tyr^3]octreotide treatment in a multicenter trial in 22 end-stage patients with progressive disease. Valkema *et al.*, *J. Nucl. Med. 41*: 111P (2000). Valkema *et al.* demonstrated that when these patients were treated with [^{90}Y-DOTA^0-Tyr^3]octreotide, a partial tumor response was observed in two patients, a minor response was observed in three patients and a stable disease was observed in ten patients.

The journal articles summarized above show the level of skill in the art at the time the application was filed. Further, the Specification, at page 44, line 4, to page 49, line 5, (i.e., Examples 10 – 12) teaches the skilled artisan (a) how to attach the radiolabel (^{99m}Tc and ^{188}Re)

to the inventive peptides and (b) protocols for, and results from *in vitro* assays using human breast adeoncarcinoma cell lines MCF-7, SK-BR-3, and MDA-MB 231 and the inventive peptides. As discussed above, the case-law and the references submitted as Exhibits A – E both establish that extrapolation from *in vitro* data to *in vivo* utility is appropriate. Therefore, based on the disclosure of the Specification and the skill in the art demonstrated in the numerous articles summarized above, Appellants submit that the ordinary skilled artisan would know how to use radiolabeled peptides, such as those disclosed and claimed in the present application, to treat tumors. Therefore, pursuant to MPEP section 2163, it is not necessary for Appellants to describe in the Specification how the claimed radiolabeled peptides would be used to treat tumors, beyond what is already described in the Specification.

X. CONCLUSION

The PTO erred when it held that the claims contain subject matter which was not described in the Specification in such a way as to reasonably convey to one skilled in the relevant art, that the inventors, at the time the application was filed, had possession of the claimed invention. Appellants have pointed to sections in the Specification where radiolabeling results and *in vitro* assays are described. Appellants have also shown that the level of skill in the art was such that it was not necessary to describe in the Specification how the radiolabeled peptides claimed in this application would be used to treat tumors. Appellants have demonstrated this by submitting various journal articles that show that the ordinary skilled artisan would know how to use radiolabeled peptides such as those disclosed and claimed in the present application to treat tumors. Accordingly, the instant Specification not only complies with the written description requirement, but it also demonstrates that Appellants had possession of the claimed invention.

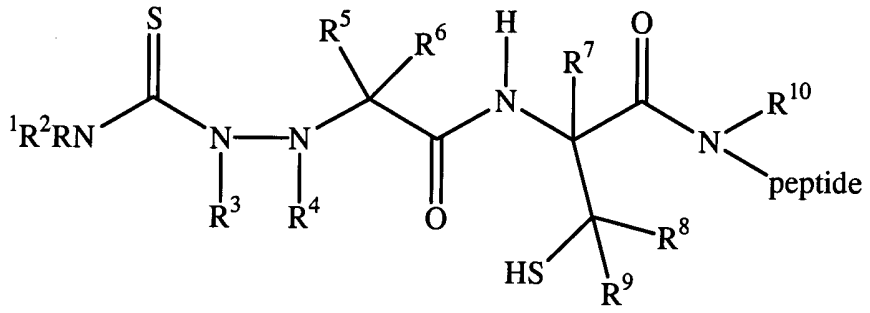
Accordingly, Appellants respectfully urge the Honorable Board of Patent Appeals and Interferences to reverse the rejection of claims 24 - 40, 42 and 43 under 35 U.S.C. § 112, first paragraph and pass this application on to allowance.

Respectfully submitted,

Stephen B. Maebius
Registration No. 35,264

XI. APPENDIX I

24. A method of treating a tumor, comprising administering to a human patient a radiolabeled peptide and a pharmaceutically acceptable carrier, wherein said peptide comprises a radiometal-binding moiety comprising the structure:



wherein R^1, R^2, and R^3 independently are selected from the group consisting of H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_3-C_6 cycloalkyl, substituted C_3-C_6 cycloalkyl, heterocycloalkyl, C_6-C_{12} aryl, C_6-C_{12} substituted aryl, heteroaryl, substituted heteroaryl, alkaryl, and a protecting group, provided that at least one of R^1, R^2, or R^3 is H,

R^5, R^7, R^8, R^9 and R^{10} independently are selected from the group consisting of H, C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_6-C_{12} aryl, and substituted C_6-C_{12} aryl, and R^8 and R^9 together or R^7 and R^9 together may form a cycloalkyl or substituted cycloalkyl ring,

R^4 and R^6 together form a direct bond or are independently selected from the group consisting of C_1-C_6 alkyl, substituted C_1-C_6 alkyl, C_6-C_{12} aryl, and substituted C_6-C_{12} aryl, and wherein NR^{10} is located at the N-terminus of said peptide, or is located on an amino acid side chain of said peptide.

25. A method according to claim 24, wherein R^1 is H.

26. A method according to claim 24, wherein R^3 is H.

27. A method according to claim 24, wherein R^4 is H.

28. A method according to claim 24, wherein R^4 and R^6 together form a direct bond.

29. A method according to claim 24, wherein R^5 is H.

30. A method according to claim 24, wherein NR^{10} is located at the N-terminus of said peptide.

31. A method according to claim 24, wherein NR^{10} is located on an amino acid side chain of said peptide.

32. A method according to claim 25, wherein R^2 is lower alkyl or substituted or unsubstituted phenyl.

33. A method according to claim 32, wherein R^2 is H.

34. A method according to claim 33, wherein R^3 is H.

35. A method according to claim 34, wherein R^4 and R^6 together form a direct bond.

36. A method according to claim 34, wherein R^5 is H.

37. A method according to claim 36, wherein R^7 , R^8, and R^9 each are H.

38. A method according to claim 37, wherein R^2 is phenyl.

39. A method according to claim 37, wherein R^2 is methyl.

40. A method according to claim 24, wherein R^8 and R^9 are methyl.

41. A method according to claim 24, wherein said peptide is selected from the group consisting of:

(Chel)γAbuNle<u>DHF$_d$ RWK</u>-NH_2, (SEQ ID NO:1)

(Chel)γAbuHSDAVFTDNYTRLRKQMAVKKYLNSILN-NH_2, (SEQ ID NO:2)

KPRRPYTDNYTRLRK(Chel)QMAVKKYLNSILN-NH_2, (SEQ ID NO:3)

(Chel)γAbuVFTDNYTRLRKQMAVKKYLNSILN-NH_2,

(Chel)γAbuYTRLRKQMAVKKYLNSILN-NH_2, (SEQ ID NO:4)

HSDAVFTDNYTRLRK(Chel)QMAVKKYLNSILN-NH_2, (SEQ ID NO:5)

(SEQ ID NO:6) <GHWSYK(Chel)LRPG-NH_2, <GHYSLK(Chel)WKPG-NH_2, (SEQ ID NO:7)

AcNal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (Chel)LRPA$_d$ -NH_2, (SEQ ID NO:8)

(SEQ ID NO:9) (Chel)γAbuSYSNleDHF$_d$ RWK-NH$_2$, Ac-HSDAVFTENYTKLRK(Chel)QNleAAKKYLNDLKKGGT-NH$_2$, (SEQ ID NO:10)

(SEQ ID NO:12) Nal$_d$ Cpa$_d$ W$_d$ SRK$_d$ (Chel)WKPG-NH$_2$, <GHWSYK$_d$ (Chel)LRPG-NH$_2$, (SEQ ID NO:13)

(SEQ ID NO:14) AcK(Chel)F$_d$ CFW$_d$ KTCT-OH, AcK(Chel)DF$_d$ CFW$_d$ KTCT-OH, (SEQ ID NO:15)

(SEQ ID NO:14) AcK(Chel)F$_d$ CFW$_d$ KTCT-ol, AcK(Chel)DF$_d$ CFW$_d$ KTCT-ol, (SEQ ID NO:15)

(SEQ ID NO:16) (Chel)DF$_d$ CFW$_d$ KTCT-OH, K(Chel)DF$_d$ CFW$_d$ KTCT-ol, (SEQ ID NO:15)

(SEQ ID NO:17) K(Chel)KKF$_d$ CFW$_d$ KTCT-ol, K(Chel)KDF$_d$ CFW$_d$ KTCT-OH, (SEQ ID NO:18)

(SEQ ID NO:19) K(Chel)DSF$_d$ CFW$_d$ KTCT-OH, K(Chel)DF$_d$ CFW$_d$ KTCT-OH, (SEQ ID NO:15)

(SEQ ID NO:20) K(Chel)DF$_d$ CFW$_d$ KTCD-NH$_2$, K(Chel)DF$_d$ CFW$_d$ KTCT-NH$_2$, (SEQ ID NO:15)

(SEQ ID NO:18) K(Chel)KDF$_d$ CFW$_d$ KTCT-NHNH$_2$, AcK(Chel)F$_d$ CFW$_d$ KTCT-NHNH$_2$, (SEQ ID NO:14)

(SEQ ID NO:14) K(Chel)F$_d$ CFW$_d$ KTCT-ol, and F$_d$ CFW$_d$ KTCTK(Chel)-NH$_2$, (SEQ ID NO:21) wherein (Chel) is a radiometal-binding moiety.

42. A method according to claim 24, wherein said peptide contains at least one disulfide bond.

43. A method according to claim 42, wherein said peptide is a polypeptide.

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1. Insert the street address of the registrant's principal place of business in Placer County. **(P.O. Box n t acceptable.)**

2. **The Fictitious Name under which business is being conducted.**

3. If the registrant is an individual, insert his full name and residence address. If the registrant is a partnership or other association of persons, insert the full name and residence address of each general partner. If the registrant is a business trust, insert the full name and residence address of each trustee. If the registrant is a corporation, insert the name of the corporation as set forth in its articles of incorporation and the State of incorporation. If the registrant is a limited liability company, insert the name stated in its articles of organization and the State of organization. (Attach additional sheet if necessary.) (P.O. Box not acceptable.)

A FICTITIOUS BUSINESS NAME STATEMENT EXPIRES FIVES YEARS FROM THE DATE IT WAS FILED IN THE OFFICE OF THE COUNTY CLERK. Except as provided in Section 17923, B&P Code, it expires 40 days after any change in the facts set forth in the statement; except that a change in the residence address of an individual, general partner, or trustee does not cause the statement to expire.

If a refiling is required because the prior statement has expired, the refiling need not be published unless there has been a change in the information required in the expired statement, **provided the refiling is fil d within 40 days of the date the statement expires.** (Sec. 17917(c) B&P Code)

NOTICE TO REGISTRANT Section 17924 Business & Professions Code
(NOTE: FIRST PUBLICATION MUST START WITHIN 30 DAYS OF COUNTY CLERK FILED DATE.)

(A) Your fictitious business name statement must be published in a newspaper once a week for four successive weeks and an affidavit of publication filed with the county clerk within 30 days after publication has been accomplished. The statement should be published in a newspaper of general circulation in the county where the principal place of business is located. The statement should be published in such county in a newspaper that circulates in the area where the business is to be conducted. (Sec. 17917 B&P Code)

(B) Any person who executes, files, or publishes any fictitious business name statement, knowing that such statement is false, in whole or in part, is guilty of a misdemeanor and upon conviction thereof shall be fined not to exceed five hundred dollars ($500). (Sec. 17930 B&P Code)

753 F.2d 1040
224 U.S.P.Q. 739
(Cite as: 753 F.2d 1040)

United States Court of Appeals,
Federal Circuit.

Peter E. **CROSS**, et al., Appellants,
v.
Kinji **IIZUKA**, et al., Appellees.

Appeal No. 84-1111.
Interference No. 100,650.

Jan. 28, 1985.

Patent and Trademark Office Board of Patent Interferences awarded priority on single phantom count relating to imidazole derivative compounds to senior party, finding him entitled to benefit of his Japanese priority application, and appeal was taken. The Court of Appeals, Kashiwa, Circuit Judge, held that: (1) Board did not err in finding that utility disclosed in Japanese priority application was sufficient to meet "practical utility" requirement; and (2) Board did not err in finding that Japanese priority application contained sufficient disclosure to satisfy enablement or "how-to-use" requirement.

Affirmed.

West Headnotes

[1] **Patents** 314(5)
291k314(5) Most Cited Cases

Whether utility disclosed in foreign priority application is sufficient to meet "practical utility" requirement for patent is fact question, whereas question of whether application contains sufficient disclosure to satisfy enablement, i.e., how-to-use requirement, is question of law. 35 U.S.C.A. § § 101, 112.

[2] **Patents** 314(5)
291k314(5) Most Cited Cases

Invention cannot be considered "useful," in sense that patent can be granted on it, unless substantial or practical utility for invention has been discovered and disclosed, where such utility would not be obvious. 35 U.S.C.A. § 101.

[3] **Patents** 90(5)
291k90(5) Most Cited Cases

Where constructive reduction to practice is involved, as contrasted to actual reduction to practice, practical utility for invention is determined by reference to, and factual analysis of, disclosures of application. 35 U.S.C.A. § 101.

[4] **Patents** 49
291k49 Most Cited Cases

Evidence of any utility of invention is sufficient to meet "practical utility" patent requirement when count does not recite any particular utility. 35 U.S.C.A. § 101.

[5] **Patents** 49
291k49 Most Cited Cases

Evidence was sufficient to sustain Board of Patent Interferences' finding that senior party's Japanese priority application sufficiently disclosed utility for imidazole derivative compounds of phantom count as inhibiting agent for thromboxane synthetase in human or bovine platelet microsomes. 35 U.S.C.A. § 101.

[6] **Patents** 49
291k49 Most Cited Cases

Every utility question arising in interference, in final analysis, must be decided on basis of its own unique factual circumstances; relevant evidence must be judged as a whole for persuasiveness in determining whether suggested use for compound of count is practical utility. 35 U.S.C.A. § 101.

[7] **Patents** 49
291k49 Most Cited Cases

In interference proceeding, evidence was sufficient to sustain Board of Patent Interferences' determination that senior party's Japanese priority application disclosed practical utility for imidazole derivatives of phantom count in inhibition of thromboxane synthetase in human or bovine platelet microsomes, i.e., in vitro utility. 35 U.S.C.A. § 101.

[8] **Patents** 99
291k99 Most Cited Cases

Board of Patent Interferences did not err in finding that senior party's Japanese priority application relating to imidazole derivatives for use as inhibiting agents for thromboxane synthetase in platelet

microsomes contained sufficient disclosure of appropriate dosage to satisfy enablement or "how-to-use" requirement. 35 U.S.C.A. § 112.

***1041** Rudolf E. Hutz, Connolly, Bove, Lodge & Hutz, Wilmington, Del., argued for appellants. With him on the brief was Thomas M. Meshbesher, Wilmington, Del.

Peter D. Olexy, Sughrue, Mion, Zinn, MacPeak & Seas, Washington, D.C., argued for appellees. With him on the brief was Thomas J. MacPeak, Washington, D.C.

Before KASHIWA, BENNETT and BISSELL, Circuit Judges.

KASHIWA, Circuit Judge.

This appeal is from the decision of the United States Patent and Trademark Office (PTO) Board of Patent Interferences (Board) awarding priority on the single phantom count to Iizuka, *et al.* (Iizuka), the senior party. We affirm.

Background

Interference No. 100,650 was declared on 20 April 1981 between application serial No. 68,365, for "Imidazole Derivatives," filed by Iizuka on 21 August 1979 and application serial No. 95,755, for "N-Phenoxyalkyl) Imidazoles as Selective Inhibitors of the Thromboxane Synthetase Enzyme and Pharmaceutical Compositions ***1042** Thereof," filed by Cross, *et al.* (Cross) on 19 November 1979. The single phantom count of the interference is directed to imidazole derivative compounds and reads as follows:

A compound selected from the group consisting of an imidazole derivative of the formula

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wherein R is a hydrogen atom or an alkyl group having 1 to 6 carbon atoms, each of A sub1 or A sub2 , which may be the same or different, are alkylene having 1 to 8 carbon atoms, m is 0 or 1, X is oxygen or sulfur, and each of R sub1 or R sub2 , which may be the same or different, is a hydrogen atom *or an alkyl group having 1 to 6 carbon atoms;* R sub3 is H, C sub1 -C sub4 alkyl, C sub1 -C sub4 alkoxy or halogen; and the pharmaceutically acceptable salts thereof. [FN1]

FN1. We note a discrepancy, shown underlined in the above count, between the phantom count as set forth by the primary examiner and that reported by the Board in its decision. The phantom count set forth herein is the one propounded by the primary examiner. However, as will become apparent from the ensuing discussion, the substance of the phantom count is not crucial to resolution of the issues presented by this case.

The applications of Cross and Iizuka both disclose inventions directed to imidazole derivative compounds which inhibit the synthesis of thromboxane synthetase, an enzyme which leads to the formation of thromboxane A sub2 (TXA sub2), [FN2] a highly unstable, biologically active compound which is converted to stable thromboxane B sub2 by the addition of water. Thromboxane A sub2 , as of the time period during which the applications were filed, was postulated to be a causal factor in platelet aggregation. [FN3] Platelet aggregation is associated with several deleterious conditions in mammalia, including humans, such as platelet thrombosis, pulmonary vasoconstriction or vasospasm, inflammation, hypertension, and collagen-induced thrombosis.

FN2. The formation of TXA sub2 in an arachidonic acid challenge is a sequential process initiated by the conversion of arachidonic acid to prostaglandin PGG sub2 by the action of cyclooxygenase, which adds oxygen to the acid. Peroxidase converts the prostaglandin PGG sub2 to prostaglandin PGH sub2 , which in turn is converted by thromboxane synthetase to TXA sub2 .

FN3. Iizuka's position is that, as of the "critical date" of his application, TXA sub2 was widely accepted in the art as causing platelet aggregation. Cross' position is that, as of the "critical date," platelet aggregation was believed to be nonspecific, i.e., platelet aggregation *may* occur in the presence of thromboxane synthetase, but thromboxane synthetase is not necessary for platelet aggregation. We note in retrospect that THE MERCK INDEX 1345-46 (10th ed.

1983) describes TXA sub2 as inducing irreversible platelet aggregation. More to the point, however, this court has noted that it is axiomatic that an inventor need not comprehend the scientific principles on which the practical effectiveness of his invention rests, nor is the inventor's theory or belief as to how his invention works a necessary element in the specification to satisfy the enablement requirement of 35 U.S.C. § 112. *Fromson v. Advance Offset Plate, Inc.*, 720 F.2d 1565, 1570, 219 USPQ 1137, 1140 (Fed.Cir.1983).

Pursuant to 37 C.F.R. § 1.231(a)(4) each party moved to be accorded the benefit of a foreign priority application under 35 U.S.C. § 119, Cross claiming priority based upon a British application filed 13 December 1978, and Iizuka claiming priority based upon a Japanese application filed 21 August 1978. Each party opposed the motion of the other party, each party contending that the other party's foreign priority application did not comply with the disclosure requirements of 35 U.S.C. § 112.

The primary examiner granted each party's motion, noting that the utility alleged in each application was of a pharmacological nature, i.e., the inhibition of thromboxane synthetase, and that inasmuch as the single phantom count of the interference was directed to a compound, it was not necessary that utility be established by tests and dosages with respect to human beings. The examiner found that one of ordinary skill in the art would know how to use the imidazole derivatives, i.e., be able to determine specific dosages, for biological purposes. Based upon the filing dates of ***1043** the foreign priority applications, [FN4] Iizuka was declared the senior party and a show cause order was issued against Cross.

FN4. Each party relies on the filing date of its foreign priority application to establish a constructive reduction to practice, the earliest date of invention to which each party is entitled under the patent laws of the United States. *Kawai v. Metlesics*, 480 F.2d 880, 885-86, 178 USPQ 158, 162 (CCPA 1973).

Cross requested a final hearing on the issue of the sufficiency of the Japanese priority application of Iizuka, and moved for a testimony period to present evidence on this issue. A testimony period was granted over the opposition of Iizuka, and Cross took the testimony of his expert witness, Dr. Smith, and Iizuka took the testimony of his expert witness, Dr. Ramwell and also proferred several exhibits pursuant to 37 C.F.R. § 1.282. All testimony and exhibits related to the sufficiency of Iizuka's Japanese priority application, i.e., whether it complied with the disclosure requirements of 35 U.S.C. § 112.

Decision of the Board

The Board noted that the sole issue before it was whether Iizuka was entitled to the benefit of his Japanese priority application. [FN5] Relying on *In re Bundy*, 642 F.2d 430, 209 USPQ 48 (CCPA 1981), and *Nelson v. Bowler*, 626 F.2d 853, 206 USPQ 881 (CCPA 1980), the Board held that tests evidencing pharmacological activity may manifest a practical utility even though they may not establish a specific therapeutic use. The Board found that the Japanese priority application disclosed pharmacological activity in the similar activity of the imidazole derivatives of the count to imidazole and 1-methylimidazole, which possess an inhibitory action for thromboxane synthetase, and that practical utility was disclosed in the strong inhibitory action for thromboxane synthetase from human or bovine platelet microsomes, i.e., an *in vitro* utility. [FN6]

FN5. More specifically, the issue before the Board was whether the Japanese priority application complied with the how-to-use requirement of 35 U.S.C. § 112. Section 112 of Title 35 provides, in pertinent part, that:

The specification shall contain a written description of the invention, of the manner and process of making and *using* it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and *use* the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention. (Emphasis added.)

Should Iizuka's Japanese priority application be found nonenabling with respect to the how-to-use requirement of § 112, or otherwise found deficient under the patent laws of the United States, priority would be awarded to Cross based upon his unchallenged entitlement to the benefit of

his British application.

FN6. Generally, *in vitro* refers to an environment outside of a living organism, usually an artificial environment such as a test tube or culture. In contradistinction, *in vivo* generally refers to an environment within a living organism, such as a plant or animal, or it may refer to a particular portion of an organ external to the living organism, e.g., rat aortic loop.

The Board further found that the Japanese priority application disclosed "how- to-use" knowledge directed to the practical utility in a microsome system, and that microsome assays were admittedly known in the art. A skilled worker could determine the relative strength of the imidazole compounds of the count vis-a-vis the known parent imidazole and 1-methylimidazole compounds for use in the microsome assay milieu. Knowledge of the pharmacological activities of compounds is beneficial to the medical profession, and requiring Iizuka to have disclosed *in vivo* dosages in the Japanese priority application would delay and frustrate researchers by failing to provide an incentive for early public disclosure of such compounds, thereby failing to further the public interest.

Accordingly, the Board held that the Japanese priority application contained an adequate how-to-use disclosure for the practical utility stated therein.

Issues

Whether the Board erred in finding that the utility disclosed in the Japanese priority application is sufficient to meet the practical utility requirement of 35 U.S.C. § 101.

***1044** [1] Whether the Board erred in finding that the Japanese priority application contained sufficient disclosure to satisfy the enablement, i.e., how-to-use, requirement of 35 U.S.C. § 112. [FN7]

FN7. Utility is a fact question. *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed.Cir.1983), *cert. denied*, 469 U.S. 835, 105 S.Ct. 127, 83 L.Ed.2d 69 (1984). Enablement under § 112, paragraph 1, i.e., the how-to-use requirement, is a question of law. *Id.* at 960 n. 6, 220 USPQ at 599 n. 6.

OPINION

Proper resolution of the issues before this court necessitates that we address, *seriatim,* the following questions: (1) What utility is disclosed by the Japanese priority application? (2) Does this stated utility comply with the "practical utility" requirement of 35 U.S.C. § 101, as delimited by prior decisions of the judiciary? [FN8] (3) Does the Japanese priority application contain sufficient disclosure to meet the how-to-use requirement of § 112 with respect to the stated utility?

FN8. While questions one and two are closely connected, a thorough analysis of the utility issue requires first, a determination as to what utility is disclosed, i.e., the stated utility, for the invention claimed in the application. Only after the stated utility has been determined, can a proper analysis be undertaken to determine if the stated utility complies with the "practical utility" requirement of § 101. As noted above, these questions regarding utility are factual in nature, *see supra* note 7, and are to be determined in the first instance by the PTO, the agency with the expertise in this regard.

[2][3] It is axiomatic that an invention cannot be considered "useful", in the sense that a patent can be granted on it, unless substantial or practical utility for the invention has been discovered and disclosed where such utility would not be obvious. *Brenner v. Manson*, 383 U.S. 519, 86 S.Ct. 1033, 16 L.Ed.2d 69, 148 USPQ 689 (1966). Where a constructive reduction to practice is involved, as contrasted to an actual reduction to practice, a practical utility for the invention is determined by reference to, and a factual analysis of, the disclosures of the application. *Kawai v. Metlesics*, 480 F.2d 880, 178 USPQ 158 (CCPA 1973).

1. Japanese Priority Application

The Board factually analyzed the Japanese priority application and found that the only effective disclosure relating to a stated utility for the imidazole derivative compounds of the phantom count was the following:

[The compounds disclosed] are useful for treatment

of inflammation, thrombus, hypertension, cerebral apoplexy, asthma, etc.

Up to this time, it is a known fact that imidazole and 1-methylimidazole possess an inhibitory action for thromboxane synthetase and inhibit a biosynthesis of thromboxane A sub2 . (*Prostaglandins,* Vol. 13, pages 611-, *1977*). However, since their inhibitory effect is not satisfactory one, these compounds have not been put to practical use yet as therapeutical medicines for diseases caused by thromboxane A sub2 , such as inflammation, hypertension, thrombus, cerebral apoplexy, asthma, etc.

To develop some compounds possessing a strong inhibitory action for biosynthesis of thromboxane A sub2 , the present inventors devoted themselves to study for various imidazole derivatives, and as a result, found that the compounds [of this invention] possess a strong inhibitory action for thromboxane synthetase from human or bovine platelet microsomes and are extremely useful as therapeutically active agents for diseases caused by thromboxane A sub2 , for example, inflammation, hypertension, thrombus, cerebral apoplexy, asthma, etc., and thus we proposed this invention based upon those findings.

* * *

The imidazole derivatives ... of this invention are novel compounds which are not described in literature, and which possess a strong inhibitory action for thromboxane synthetase from human or bovine platelet microsomes, and which ***1045** exhibit a strong inhibitory action for biosynthesis of thromboxane A sub2 in mammalia including human. In general, a satisfactory inhibitory effect is found at a level of molar concentrations of 2.5 x 10-8, for example, 2-[p-(1-imidazolylmethyl) phenoxy]-acetic acid hydrochloride produce the about 50% inhibitory effect at the molar concentrations of 2.5 x 10-8. Accordingly, the imidazole derivatives of this invention are extremely useful as therapeutical medicines for diseases caused by thromboxane A sub2 , such as inflammation, hypertension, thrombus, cerebral apoplexy, asthma, etc.

The Board found that these pertinent sections of the Japanese priority application disclosed some activity or utility, namely that the imidazole derivative compounds of the count possess a strong inhibitory action for thromboxane synthetase in human or bovine platelet microsomes. Cross' position is that the stated purpose or *sole* contemplated utility of the invention of Iizuka is to provide a novel class of compounds which provide "practical use" as "therapeutical medicines for diseases caused by thromboxane A sub2 ," and therefore the Board erred in its finding as to the stated utility of the Japanese priority application.

While recognizing that *Kawai* constrains an applicant to entitlement to the benefit of only what is disclosed in the foreign priority application and no more, we also recognize that foreign priority applications, as subsequently filed in the PTO, typically have a style and format dissimilar to the arrangement of application elements suggested by 37 C.F.R. § 1.77. In part this arises because of differences in filing requirements in foreign patent offices, and in part because of the awkwardness resulting from direct literal translations from a foreign language to English. Thus, while the factual determination of the stated utility in an application prepared in the United States may be relatively straightforward, [FN9] the factual analysis of a foreign priority application to determine the utility disclosed therein may be more laborious and open to varying interpretations.

FN9. In applications prepared in the United States by experienced patent drafters, the drafter of the application typically sets forth objectives for the invention in the "Summary of the Invention" section of the application. These objectives will normally be consonant with the utility disclosed for the invention. As this court has noted, "[w]hen a properly claimed invention meets at least one stated objective, utility under § 101 is clearly shown." *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 958, 220 USPQ 592, 598 (Fed.Cir.1983), *cert. denied,* 469 U.S. 835, 105 S.Ct. 127, 83 L.Ed.2d 69 (1984).

The weakness of Cross' position is that a fair reading of the pertinent sections of the Japanese priority application, as set forth above, discloses utility for the imidazole derivative compounds of the phantom count both as an inhibiting agent for thromboxane synthetase in human or bovine platelet microsomes, as found by the Board, and as therapeutically active agents preventing the biosynthesis of thromboxane A sub2 , thereby functioning as a medicine preventing deleterious conditions caused by thromboxane A sub2 , as contended by Cross.

[4][5] Evidence of any utility is sufficient when the count does not recite any particular utility. *Nelson v. Bowler*, 626 F.2d 853, 856, 206 USPQ 881, 883 (CCPA 1980). *See also Rey-Bellet v. Englehardt*, 493 F.2d 1380, 181 USPQ 453 (CCPA 1974); *Knapp v. Anderson*, 477 F.2d 588, 177 USPQ 688 (CCPA 1973); *Blicke v. Treves*, 241 F.2d 718, 112 USPQ 472 (CCPA 1957). Here the Board, which is charged with the factual determination of utility, [FN10] has found that the specification of the Japanese priority application disclosed *a* utility for the imidazole derivative compounds of the phantom count in the inhibition of thromboxane ***1046** synthetase in human or bovine platelet microsomes. Inasmuch as the Board is charged with making this factual determination when the issue is raised, inasmuch as they have so done in the instant case, and inasmuch as there is credible evidence to support this factual determination, we are not prepared to say that the Board erred in its finding as to the stated utility disclosed in the Japanese priority application.

FN10. Under the facts of the instant case, utility and enablement questions are ancillary to priority. In the interference proceeding, Cross raised the issue as to whether the Japanese priority application contained sufficient disclosure to satisfy § 112. As noted above, *see supra* note 5, if Cross prevails on this issue the Japanese priority application would be removed as the basis for awarding priority to Iizuka. *See generally* 37 C.F.R. § § 1.225, .231, .258.

2. Practical Utility

As noted in the preceding part of this opinion, Cross has contended that the Board erred in its finding as to the utility disclosed in the Japanese priority application. This argument may be viewed in a different perspective, we believe, which is that the stated utility in the Japanese priority application, as found by the Board--the inhibition of thromboxane synthetase in human or bovine platelet microsomes [FN11]--is not sufficiently correlated to a pharmacological activity [FN12] to be a practical utility. In other words, Cross may be arguing that the minimum acceptable level of utility disclosed in an application claiming a compound having pharmacological activity must be directed to an *in vivo* utility in order to comply with the practical utility requirement of § 101.

FN11. A platelet microsome is an *in vitro* milieu consisting of blood platelets, the small, colorless corpuscles in the blood of all mammals, and other finely granular elements of protoplasm, such as ribosomes, fragmented endoplasmic reticula and mitochondrial christae.

FN12. Generally, pharmacological activity refers to the properties and reactions of drugs, especially with relation to their therapeutic value.

The starting point for a practical utility analysis is *Brenner v. Manson*, 383 U.S. 519, 86 S.Ct. 1033, 16 L.Ed.2d 69, 148 USPQ 689 (1966). The Court in *Brenner* noted that "a simple, everyday word ["useful," as found in 35 U.S.C. § 101] can be pregnant with ambiguity when applied to the facts of life." *Id.* at 529, 86 S.Ct. at 1039, 148 USPQ at 693. While noting that "one of the purposes of the patent system is to encourage dissemination of information concerning discoveries and inventions," *id.* at 533, 86 S.Ct. at 1041, 148 USPQ at 695, the Court found that a more compelling consideration in the determination of whether a patent should be granted "is the benefit derived by the public from an invention with substantial utility. Unless and until a process is refined and developed to this point--where specific benefit exists in currently available form--there is insufficient justification for permitting an applicant to engross what may prove to be a broad field." *Id.* at 534-35, 86 S.Ct. at 1042, 148 USPQ at 695. While we recognize that this case concerned a compound derived from a chemical process, we believe *Brenner* provides broad guidelines which are helpful in ascertaining what constitutes practical utility for compounds having a pharmacological effect.

In *Nelson v. Bowler*, 626 F.2d 853, 206 USPQ 881 (1980), our predecessor court, the Court of Customs and Patent Appeals, stated that "[k]nowledge of the pharmacological activity of any compound is obviously beneficial to the public" and concluded that "adequate proof of any such utility constitutes a showing of practical utility." *Id.* at 856, 206 USPQ at 883. [FN13] The tests [FN14] found by the court to be adequate proof of pharmacological activity or practical utility were a rat blood pressure (BP) test and a gerbil colon smooth muscle stimulation (GC-SMS) test. The BP test was an *in vivo* test, which was deemed by the court to be direct evidence as to

the claimed ***1047** activity, while the GC-SMS test was an *in vitro* test. [FN15]

FN13. For purposes of the present opinion, we consider the phrase "substantial utility," as enunciated in *Brenner,* to be synonymous with the phrase "practical utility" as used in subsequent opinions of the CCPA.

FN14. We recognize that *Nelson* dealt with tests which were found adequate to establish an actual reduction to practice, as opposed to a constructive reduction to practice. We agree with the Board that principles applicable to a determination of an actual reduction to practice are generally germane to a constructive reduction to practice.

FN15. Both parties admitted that the GC-SMS test adequately simulated *in vivo* smooth muscle stimulation.

The CCPA in *Rey-Bellet v. Englehardt,* 493 F.2d 1380, 1383, 181 USPQ 453, 454 (1974), stated that where a count contains no limitation related to utility, evidence establishing a substantial utility for any purpose is sufficient to show a reduction to practice. The court held that three *in vivo* tests [FN16] conducted in the United States prior to the filing of Englehardt's U.S. application failed to establish an actual reduction to practice. The court proceeded, however, to find sufficient evidence in the record to establish that Englehardt had conceived a utility for his compound prior to the filing date of his U.S. application. The evidence the court found to be sufficient was testimony by the inventor that he believed his compound would exhibit a particular pharmacological activity because of its structural similarity to another compound which was known to possess the particular pharmacological activity. The court found that the testimonial evidence of Englehardt was corroborated by two exhibits entered into evidence. The evidence adduced by Englehardt was found by the court to be sufficient proof that Englehardt had conceived that his compound had utility for the particular pharmacological activity prior to his U.S. filing date. The court further noted that this was a completed conception of utility because it appeared that nothing beyond the exercise of routine skill would have been required to demonstrate that Englehardt's compound possessed the particular pharmacological utility. While noting that the actual testing done was not sufficient to establish an actual reduction to practice, the court found that the extensive testing done *in vivo* on animals was routine in nature and was not, therefore, to be construed as an indicator that extensive research, i.e., inventive skill and/or undue experimentation, was required to resolve perplexing intricate difficulties related to the utilization of the compound for the particular pharmacological activity.

FN16. The three tests, all *in vivo* type tests carried out on laboratory animals, were: (1) the Mental Health General Screening Test which indicated the physical response, or absence of a response, of test animals to a drug, indicating the presence, or absence, of a desired pharmacological activity; (2) the Tetrabenazine Antagonism Test which screened drugs for antidepressant activity; and (3) the Sidman Avoidance Test which screened drugs for tranquilizing activity.

The CCPA in *Kawai v. Metlesics,* 480 F.2d 880, 178 USPQ 158 (1973), concurred with the finding of the Board that the applicants had failed to prove that their foreign priority application was adequate under the patent laws of the United States. The only disclosure in the foreign priority application relating to the compound of the count was that it exhibited "pharmacological effects on the central nervous system," which the applicants conceded was an inadequate disclosure. The applicants, however, relied upon a patent made of record as indicative of the general knowledge of one skilled in the art, which the applicants contended described a compound closely related to their claimed compound, to show utility or pharmacological activity for the compound of the count as an anticonvulsant. The court agreed with the board that there were sufficient structural dissimilarities between the compounds of the patent and those of the count to preclude reliance on the patent to supplement the disclosure deficiencies of the foreign priority application.

In *Knapp v. Anderson,* 477 F.2d 588, 177 USPQ 688 (CCPA 1973), the court, citing to *Blicke v. Treves,* 241 F.2d 718, 112 USPQ 472 (CCPA 1957), stated that "[i]t is well settled that if the counts do not specify any particular use, evidence proving *substantial utility for any purpose* is sufficient to establish an actual reduction to practice." *Id.* 477

F.2d at 590, 177 USPQ at 690 (emphasis added). Noting that the only utility contemplated for the compounds of the count was as ashless dispersants in lubricant compositions used in internal combustion engines, the court found no error in the Board's holding that there was no actual reduction to practice because ***1048** only a potential utility had been established, this holding based upon the Board's finding of a lack of correlation between bench tests and actual service conditions in a combustion engine.

The CCPA has held that nebulous expressions, such as "biological activity" or "biological properties," disclosed in a specification convey little explicit indication regarding the utility of a compound. *In re Kirk*, 376 F.2d 936, 941, 153 USPQ 48, 52 (CCPA 1967). But, while agreeing with the Board that the specification failed to disclose a specific allegation of utility for any compound within the scope of the claims, and that reference in the specification to biological properties of the claimed compound was so general and vague as to be meaningless, the court implied that a disclosure in the specification that the requisite properties of the claimed compounds are similar to those of a natural or synthetic hormone of known activity may, in appropriate circumstances, supplement an application to rectify an inadequate disclosure relating to the practical utility for the compound. *Id.* at 942, 153 USPQ at 53.

[6] Every utility question arising in an interference, in the final analysis, must be decided on the basis of its own unique factual circumstances. Relevant evidence must be judged as a whole for its persuasiveness in determining whether the suggested use for the compound of the count is a practical utility. *Cf. Nelson*, 626 F.2d at 858, 206 USPQ at 885.

[7] The Board has found that the Japanese priority application of Iizuka disclosed a practical utility for the compounds of the phantom count in the inhibition of thromboxane synthetase in human or bovine platelet microsomes, i.e., an *in vitro* utility. Clearly, this stated utility as found by the Board has been delimited with sufficient specificity to satisfy the threshold requirements of *Kawai* and *Kirk*. The stated utility of the Japanese priority application is directed to a specific pharmacological activity possessed by the imidazole derivatives of the phantom count--the inhibition of thromboxane synthetase *in vitro*. Thus, this court on review is not presented with a general allegation of "biological activity" or "biological properties" as was the CCPA in *Kirk*, nor is reliance on prior art required to ascertain what specific pharmacological activity the compound of the count possesses, the factual situation confronting the court in *Kawai*.

The Japanese priority application, moreover, disclosed that it was generally known in the art, as of the critical date, that the parent imidazole and 1-methylimidazole compounds possessed an inhibitory action for thromboxane synthetase. Reliance on this disclosure in the specification of the pharmacological property of the parent imidazole and 1-methylimidazole compounds, as going towards proof of the pharmacological activity of the imidazole derivatives of the phantom count, is particularly relevant in the instant case, we believe, because Iizuka is not relying on this inference to supplement an inadequate disclosure in the Japanese priority application regarding the pharmacological activity of the compound of the phantom count, but rather is relying on this inference as cumulative probative evidence showing an adequately disclosed practical utility in the Japanese priority application.

This court, in *Rey-Bellet* and *Kawai*, has implied that a particular pharmacological activity identified with prior art compounds may have probative value as to the fact that the compound of the count possesses this particular pharmacological activity where there is a structural similarity between the prior art compounds and the compound of the count. *Rey-Bellet*, 493 F.2d at 1385-87, 181 USPQ at 456-58; *Kawai*, 480 F.2d at 890-91, 178 USPQ at 166- 67. Cross has failed to proffer sufficient evidence or present any persuasive arguments going to the question of significant structural dissimilarities between the parent imidazole and 1-methylimidazole compounds and the imidazole derivatives of the phantom count. [FN17]

FN17. Contrary to Cross' contention in the Reply Brief, the evidence of record relied upon by Cross to show significant structural dissimilarity appears to us to be directed to the fact that there is a wide disparity in potency for thromboxane synthetase inhibition between the parent imidazole compound and prior art imidazole derivatives. Cross has not directed our attention to any specific evidence of record which establishes, or tends to establish, significant structural dissimilarities between the basic imidazole compound and the imidazole derivatives of the phantom count. Variation in potency, moreover, is a matter of degree of activity, *see Bundy*, 642 F.2d at

433, 209 USPQ at 51, but is still indicative of activity. There is no requirement that the compounds have the same degree of activity. *Id.*, 209 USPQ at 51. Moreover, this argument may be construed as a tacit admission that the parent imidazole compound does possess the particular pharmacological activity of inhibiting thromboxane synthetase.

Along this line, we note that Dr. Smith, Cross' expert witness, testified generally, based upon the exhibits proffered by Iizuka, *see infra* note 18, that the parent imidazole compound possessed pharmacological activity for inhibiting thromboxane synthetase, although stating that there was a wide potency spectrum for prior art imidazole derivatives with respect to the parent imidazole compound.

Cross has directed the court's attention to the fact that the Japanese priority application, while disclosing that the parent imidazole and 1- methylimidazole compounds possess an inhibitory action for thromboxane synthetase, further discloses that this inhibitory effect is not satisfactory and that the parent imidazole and 1-methylimidazole compounds have not been put to practical therapeutic use. But a therapeutical utility is not necessarily synonymous to a pharmacological activity. *Cf.* Nelson, 626 F.2d at 856, 206 USPQ at 883.

***1049** The expert of Iizuka, Dr. Ramwell, testified that, as of the critical date, there was an awareness on the part of those skilled in the art that the parent imidazole compound exhibited an inhibitory activity for thromboxane synthetase, in both *in vitro* and *in vivo* environments. Dr. Ramwell further testified that there was an awareness by those skilled in the art of a correlation between thromboxane A sub2 and platelet aggregation, namely that thromboxane A sub2 was a mediator in platelet aggregation. Several exhibits proferred by Iizuka corroborated Dr. Ramwell's testimony as to the general knowledge in the art with respect to the inhibitory effect of the parent imidazole compound for thromboxane synthetase. [FN18] Accordingly, the similar pharmacological activity of the parent imidazole and 1-methylimidazole compounds have probative value in the factual determination of practical utility for the compounds of the phantom count inasmuch as Cross has not met the burden of proof to establish structural dissimilarities between the parent imidazole and 1-methylimidazole compounds and the imidazole derivatives of the phantom count. Rey-Bellet, 493 F.2d at 1386-87, 181 USPQ at 457.

FN18. For example, Table I in the article "*Imidazole:* A Selective Inhibitor of Thromboxane Synthetase," PROSTAGLANDINS, Vol. 13, No. 4, April 1977 (Iizuka Exhibit No. 6), lists 1-methylimidazole and the parent imidazole compounds as possessing inhibitory activity for thromboxane synthetase, thereby offering corroboration of Dr. Ramwell's testimony.

The Board noted that Iizuka Exhibits 2-6 and 10-12, while inadmissible for the purpose of establishing the truth of what they say on their face, are admissible to bolster and support the testimony of Dr. Ramwell, as well asfor the purpose of establishing what literature was available to the art at the critical time. Thus, for review purposes, we have examined these exhibits for their corroborating value with respect to Dr. Ramwell's testimony.

The Board found that there was adequate proof that the Japanese priority application disclosed a pharmacological activity for the compounds of the phantom count in inhibiting the action of thromboxane synthetase, similar to the pharmacological activity of the parent imidazole and 1-methylimidazole compounds which were found to possess an inhibitory action for thromboxane synthetase, this disclosed knowledge of the inhibitory action of the prior art compounds having been corroborated by testimony and documentary evidence. During the proceedings before the Board, the burden of proof rested upon Cross to show that the Japanese priority application was deficient. 37 C.F.R. § 1.257(a). On review, Cross bears the burden of proof to show that the Board erred in finding that the Japanese priority application had adequately disclosed a practical utility. Reviewing the relevant evidence presented to the Board as a whole, we are not persuaded that Cross has met this burden of proof.

***1050** The final question we must address is whether the inhibitory activity for thromboxane synthetase in human or bovine platelet microsomes, i.e., an *in vitro* utility, is sufficient to comply with the practical utility requirement of § 101. Based upon the facts

of this case, we are not persuaded that the Board erred in finding that the *in vitro* utility disclosed in the Japanese priority application for the compounds of the count is sufficient to establish a practical utility.

Our predecessor court has noted that adequate proof of any pharmacological activity constitutes a showing of practical utility. *See, e.g.,* Nelson, 626 F.2d at 856, 206 USPQ at 883; *Rey-Bellet,* 493 F.2d at 1383, 181 USPQ at 454. Dr. Ramwell testified that initial testing of compounds for a particular pharmacological activity is typically done *in vitro*. *In vitro* testing permits an investigator to establish the rank order of compounds with respect to the particular pharmacological activity, i.e., to determine the relative potency of the compounds. Compounds having the highest ranking or potency are then selected for further testing *in vivo*. Presumably this is the accepted practice in the pharmaceutical industry inasmuch as Cross has not proffered any evidence refuting this testimony of Dr. Ramwell, and we note that this practice has an inherent logical persuasiveness. *In vitro* testing, in general, is relatively less complex, less time consuming, and less expensive than *in vivo* testing. Moreover, *in vitro* results with respect to the particular pharmacological activity are generally predictive of *in vivo* test results, i.e., there is a reasonable correlation therebetween. Were this not so, the testing procedures of the pharmaceutical industry would not be as they are. Iizuka has not urged, and rightly so, that there is an invariable exact correlation between *in vitro* test results and *in vivo* test results. Rather, Iizuka's position is that successful *in vitro* testing for a particular pharmacological activity establishes a significant probability that *in vivo* testing for this particular pharmacological activity will be successful.

As discussed above, Dr. Ramwell testified that the parent imidazole and 1- methylimidazole compounds had been subjected to both *in vitro* and *in vivo* testing as of the critical date, this corroborated by documentary evidence, and found to possess an inhibitory effect for thromboxane synthetase. Based upon this, Dr. Ramwell further testified that he would expect that *in vivo* testing of the imidazole derivatives of the phantom count would show that these compounds also possessed an inhibitory action for thromboxane synthetase, i.e., there would be a reasonable correlation between *in vitro* test results and *in vivo* test results. This evidence was found sufficient by the Board as proof that the Japanese priority application had disclosed a completed practical utility for the imidazole derivatives of the phantom count in inhibiting thromboxane synthetase in human or bovine platelet microsomes. *Cf.* Rey-Bellet, 493 F.2d at 1386-87, 181 USPQ at 457.

Cross argues that the *in vitro* utility disclosed by the Japanese priority application is not *per se* useful, and that more sophisticated *in vitro* tests, using intact cells, or *in vivo* tests are necessary to establish a practical utility. [FN19] Cross is arguing that there must be a rigorous correlation of pharmacological activity between the disclosed *in vitro* utility and an *in vivo* utility to establish a practical utility. We, however, find ourselves in agreement with the Board that, based upon the relevant evidence as a whole, there is a reasonable correlation between the disclosed *in vitro* utility and an *in vivo* activity, and therefore a rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence. *Cf.* Nelson, 626 F.2d at 856, 206 USPQ at 883-83.

> FN19. Cross is seemingly arguing that the *in vitro* disclosure of the Japanese priority application is only a *potential* utility. *See* Knapp v. Anderson, 477 F.2d 588, 591, 177 USPQ 688, 691 (CCPA 1973).

Our predecessor court has accepted evidence of *in vivo* utility as sufficient to ***1051** establish a practical utility. *See, e.g.,* Nelson v. Bowler, 626 F.2d 853, 206 USPQ 881 (CCPA 1980); *In re Jolles,* 628 F.2d 1322, 206 USPQ 885 (CCPA 1980); *Rey-Bellet v. Englehardt,* 493 F.2d 1380, 181 USPQ 453 (CCPA 1974).

Opinions of our predecessor court have recognized the fact that pharmacological testing of animals is a screening procedure for testing new drugs for practical utility. *See, e.g., In re Jolles,* 628 F.2d 1322, 1327, 206 USPQ 885, 890 (CCPA 1980). This *in vivo* testing is but an intermediate link in a screening chain which may eventually lead to the use of the drug as a therapeutic agent in humans. We perceive no insurmountable difficulty, under appropriate circumstances, in finding that the first link in the screening chain, *in vitro* testing, may establish a practical utility for the compound in question. Successful *in vitro* testing will marshal resources and direct the expenditure of effort to further *in vivo* testing of the most potent compounds, thereby providing an immediate benefit to the public, analogous to the benefit provided by the showing of

an *in vivo* utility. *Cf.* Nelson, 626 F.2d at 856, 206 USPQ at 883.

Today, under the circumstances of the instant case, where the Japanese priority application discloses an *in vitro* utility, i.e., the inhibition of thromboxane synthetase in human or bovine platelet microsomes, and where the disclosed *in vitro* utility is supplemented by the similar *in vitro* and *in vivo* pharmacological activity of structurally similar compounds, i.e., the parent imidazole and 1-methylimidazole compounds, we agree with the Board that this *in vitro* utility is sufficient to comply with the practical utility requirement of § 101.

3. Enablement

[8] The Board found that the knowledge as to the use of the pharmacological activity disclosed in the Japanese priority application lay in the fact that the system was a microsome system, microsome systems admittedly being known to those skilled in the art. Employing a microsome assay, the skilled worker could determine the relative strength of the compounds of the count vis-a-vis the known parent imidazole and 1-methylimidazole compounds. Thus, the dosage in the microsome assay milieu could be determined without inventive skill or undue experimentation.

Since we have agreed with the Board that the practical utility for the imidazole derivatives of the phantom count lies in their pharmacological activity in the microsome environment, the how-to-use requirement of § 112 must be analyzed with reference to the microsome environment. We are confronted with a disclosure, similar to the situation before the court in *Bundy,* that fails to reveal dosages for the novel compounds *per se.* 642 F.2d at 434, 209 USPQ at 51. Although the Japanese priority application does disclose the fact that the imidazole derivatives of the phantom count possess a pharmacological activity similar to the parent imidazole and 1-methylimidazole compounds, the priority application, unlike the application in *Bundy,* does not disclose dosages for the parent imidazole and 1-methylimidazole compounds.

We agree with the Board, however, that this deficiency in the Japanese priority application is not fatal. The testimonial evidence of Dr. Ramwell, corroborated by certain documentary evidence, showed that those skilled in the art had available, at the critical date, information as to approximate dosage levels for the parent imidazole and 1-methylimidazole compounds to produce an I subC50 effect, i.e., a 50% inhibition of thromboxane synthetase, in a microsome milieu. The objective of the pharmaceutical research undertaken by the parties was to discover imidazole derivatives having a potent inhibitory effect for thromboxane synthetase. Therefore, we believe it is logical, as did the Board, that the starting point for determining I subC50 dosage levels for the imidazole derivatives of the phantom count would be the I subC50 dosage levels of the parent imidazole and 1-methylimidazole compounds. The Board found that there was sufficient credible evidence that one skilled in the art, without the exercise of ***1052** inventive skill or undue experimentation, could determine the I subC50 dosage level for the imidazole derivatives of the phantom count in the microsome environment. *Cf.* *Bundy, id.,* 209 USPQ at 51. We do not believe the Board erred in arriving at this conclusion. This is not a case such as *In re Gardner,* 427 F.2d 786, 166 USPQ 138 (1970), where the CCPA held that the applicant's disclosure was nonenabling because inventive skill and undue experimentation would be required to discover appropriate dosages for humans, i.e., a therapeutic use. In the instant case, we are confronted with a pharmacological activity or practical utility, not a therapeutic use.

While we agree with the Board that the disclosure in the Japanese priority application is somewhat confusing with respect to the 2.5 x 10-8 level of molar concentrations, and that the 2-[p-(1-imidazolylmethyl)phenoxyl]-acetic acid hydrochloride compound is outside the phantom count of the interference, this disclosed molar concentration, we believe, does provide some probative value going towards the sufficiency of the Japanese priority application for an enabling disclosure. The disclosed molar concentration would provide sufficient information as to an initial dosage level so that one skilled in the art could determine, without inventive skill or undue experimentation, the necessary molar concentrations for the imidazole derivatives of the phantom count to achieve the desired pharmacological effect, i.e., the 50% inhibition of thromboxane synthetase in human or bovine platelet microsomes.

The Board held the disclosure of the Japanese priority application adequate to satisfy the first paragraph of § 112. The burden is on Cross to show Board error in arriving at this conclusion, and we are not persuaded that Cross has successfully carried this burden. Accordingly, we are satisfied that the how-to-use requirement of § 112 has been complied with

by the disclosures of the Japanese priority application.

AFFIRMED.

753 F.2d 1040, 224 U.S.P.Q. 739

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[CANCER RESEARCH 58, 437–441, February 1, 1998]

Comparison of ^{111}In-labeled Somatostatin Analogues for Tumor Scintigraphy and Radionuclide Therapy[1]

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ABSTRACT

We evaluated the following ^{111}In-labeled somatostatin (SS) analogues (diethylenetriaminepentaacetic acid, DTPA; tetraazacyclododecanetetraacetic acid, DOTA): [DTPA^0]octreotide, [DTPA^0,Tyr^3]octreotide, [DTPA^0,D-Tyr^1]octreotide, [DTPA^0,Tyr^3]octreotate [Thr(ol) in octreotide replaced with Thr], and [DOTA^0,Tyr^3]octreotide, *in vitro* and *in vivo*.

***In vitro*, all compounds showed high and specific binding to SS receptors in mouse pituitary AtT20 tumor cell membranes, and IC_{50}s were in the nanomolar range. Furthermore, all compounds showed specific internalization in rat pancreatic tumor cells; uptake of [^{111}In-DTPA^0,Tyr^3]octreotate was the highest of the compounds tested, and that of [^{111}In-DTPA^0,D-Tyr^1]octreotide was the lowest. Biodistribution experiments in rats showed that, 4, 24, and 48 h after injection of [^{111}In-DTPA^0,Tyr^3]octreotide, [^{111}In-DTPA^0,Tyr^3]octreotate, and [^{111}In-DOTA^0,Tyr^3]octreotide, radioactivity in the octreotide-binding, receptor-expressing tissues and tumor-to-blood ratios were significantly higher than those after injection of [^{111}In-DTPA^0]octreotide. Uptake of [^{111}In-DTPA^0,Tyr^3]octreotate in the target organs was also, *in vivo*, the highest of the radiolabeled peptides tested, whereas that of [^{111}In-DTPA^0,D-Tyr^1]octreotide was the lowest. Uptake of [^{111}In-DTPA^0,Tyr^3]octreotide, [^{111}In-DTPA^0,Tyr^3]octreotate, and [^{111}In-DOTA^0,Tyr^3]octreotide in target tissues was blocked by >90% by 0.5 mg of unlabeled octreotide, indicating specific binding to the octreotide receptors. Blockade of [^{111}In-DTPA^0,D-Tyr^1]octreotide was >70%. In conclusion, radiolabeled [DTPA^0,Tyr^3]octreotide and, especially, [DTPA^0,Tyr^3]octreotate and their DOTA-coupled counterparts are most promising for scintigraphy and radionuclide therapy of SS receptor-positive tumors in humans.**

INTRODUCTION

Radiolabeled tumor receptor-binding peptides can be used for *in vivo* scintigraphic imaging. An example is SS,[3] which binds to its receptors on tumors of neuroendocrine origin (1). The native peptide, however, is susceptible to very rapid enzymatic degradation (2) and is, therefore, not useful for *in vivo* application. Therefore, more stable synthetic SS analogues have been developed; *e.g.*, the octapeptide octreotide (Fig. 1; Ref. 3). Because octreotide cannot be radiolabeled easily with a γ-emitting radionuclide, Tyr^3-octreotide was developed, allowing radioiodination of the molecule (Fig. 1). This compound, radiolabeled with ^{125}I or ^{123}I, was the first used in *in vitro* SS receptor studies (4), tumor scintigraphy in animals (4), and in humans (1, 5). [^{111}In-DTPA^0]octreotide, consisting of the octapeptide octreotide and the chelator DTPA (Fig. 1), enabling radiolabeling with a radiometal like ^{111}In, was the next SS analogue to be synthesized for scintigraphy of SS receptor-positive lesions *in vivo*. We have described its advantages over radioiodinated Tyr^3-octreotide and its use for scintigraphic imaging of SS receptor-positive lesions (6, 7).

A new and fascinating application is the use of radiolabeled octreotide for radionuclide therapy. Promising results with regard to tumor growth inhibition have been reported in humans using [^{111}In-DTPA^0]octreotide (8). A β^- particle emitter, such as ^{90}Y, may, in certain cases, appear more suitable for this purpose than the Auger electron emitter ^{111}In. However, ^{90}Y-DTPA is unstable, resulting in hematopoietic toxicity *in vivo*; therefore, Tyr^3-octreotide has been derivatized with the DOTA chelator (Fig. 1), enabling stable radiolabeling with ^{90}Y and ^{111}In. (Pre)clinical studies with [DOTA^0,Tyr^3]octreotide showed favorable biodistribution and tumor uptake characteristics (9–11).

The success of the therapeutic strategy relies upon the amount of radioligand, which can be concentrated within tumor cells, and this will, among other things, be determined by the rates of internalization, degradation, and recycling of both ligand and receptor. We have evaluated and compared the different mentioned ^{111}In-chelator-peptide constructs, and we have also studied some new, recently synthesized SS analogues, with regard to binding to octreotide receptors on mouse pituitary tumor cell membranes and internalization in rat pancreatic tumor cells. Furthermore, biodistribution in tumor-bearing rats was investigated *in vivo*. The newly synthesized analogues tested were [DTPA^0,D-Tyr^1]octreotide and [DTPA^0,Tyr^3]octreotate (structures shown in Fig. 1).

MATERIALS AND METHODS

Labeling of Octreotide Derivatives. [DTPA^0]octreotide and $^{111}InCl_3$ were provided by Mallinckrodt Medical (Petten, the Netherlands), and octreotide was supplied by Sandoz (Basel, Switzerland). [DOTA^0,Tyr^3]octreotide was synthesized by H. R. M., and [DTPA^0,D-Tyr^1]octreotide, [DTPA^0,Tyr^3]-octreotide, and [DTPA^0,Tyr^3]octreotate were synthesized by A. S. ^{111}In labeling of the DTPA-analogues was as described for [DTPA^0]octreotide (12), and ^{111}In-labeling of [DOTA^0,Tyr^3]octreotide (9) and ^{125}I-labeling of [Tyr^3]octreotide (4) were performed as described.

***In Vitro* Receptor Binding Studies.** Receptor binding assays were carried out using [^{125}I-Tyr^3]octreotide (2200 Ci/mmol) as radioligand using mouse AtT20 pituitary tumor cell membranes (13).

Internalization. AR42J cells were grown in RPMI 1640 (Life Technologies, Inc., Grand Island, NY), CA20948 cells were grown in DMEM (Life Technologies, Inc.), and ARO cells were grown in DMEM/F12 (Life Technologies, Inc.); for all cell lines, medium was supplemented with 2 mM glutamine and 10% FCS. Before the experiment, subconfluent cell cultures were transferred to six-well plates.

The binding of the radiolabeled peptides to tumor cells and subsequent internalization were studied essentially as described (14). In short, before the experiments, cells were washed, and incubation was started by addition of 1 ml of internalization medium/well (culture medium without FCS but with 1% BSA) with 80 kBq of peptide (0.1 nM concentration). Cells were incubated at 37°C for indicated periods of time. To determine nonspecific internalization, cells were incubated with an excess unlabeled octreotide (0.1 μM). Cellular

Received 8/6/97; accepted 12/3/97.

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[1] This work was supported by Swiss National Science Foundation Grant 31-42516/94 and the "Regionale Krebsliga beider Basel" (both to H. R. M.).

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[3] The abbreviations used are: SS, somatostatin; DTPA, diethylenetriaminepentaacetic acid; DOTA, tetraazacyclododecanetetraacetic acid; AUC, area under the curve; %ID, percentage injected dose.

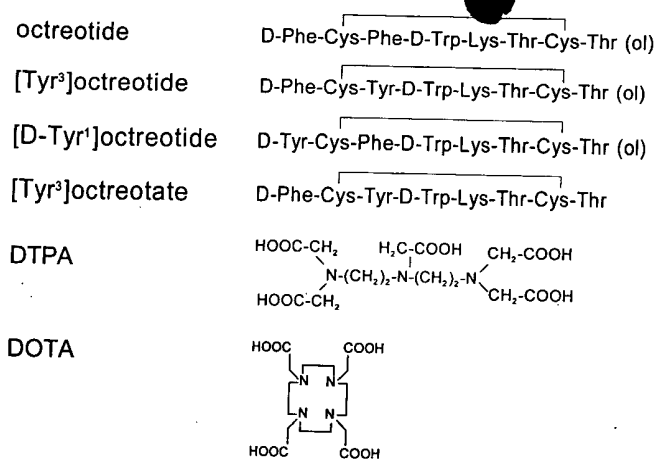

Fig. 1. Structures of octreotide, D-Tyr¹-octreotide, Tyr³-octreotide, Tyr³-octreotate, DTPA, and DOTA.

Table 1 *IC_{50} of unlabeled peptides*

Binding of [^{125}I-Tyr³]octreotide to mouse AtT20 pituitary cell membranes. Results are means of triplicate measurements in a representative experiment.

Unlabeled peptide	IC_{50} (nM)
[DTPA⁰]octreotide	3
[DTPA⁰,Tyr³]octreotide	3.2
[DTPA⁰,D-Tyr¹]octreotide	6.3
[DTPA⁰,Tyr³]octreotate	3.6
[DOTA⁰,Tyr³]octreotide	0.6

uptake was stopped by removing medium from the cells and washing with 2 ml of ice-cold PBS. To discriminate between internalized and noninternalized (surface-bound) radiopharmaceuticals, intact cells were incubated with 1 ml of 20 mM sodium acetate. The internalized and noninternalized fractions were determined by measuring radioactivity in a LKB-1282-Compugammasystem. The internalized fraction was expressed as percentage of the applied dose per mg of cellular protein. The latter was determined using a commercially available kit (Bio-Rad, Veenendaal, the Netherlands).

***In Vivo* Tissue Distribution.** Animal experiments were performed in compliance with regulations of our institution and with generally accepted guidelines governing such work. Male Lewis rats, bearing the CA20948 pancreatic tumor or Wistar male rats (200–250 g) were used in the experiments. Rats were injected under ether anesthesia with 3 MBq (0.5 μg) of ^{111}In-labeled peptide in 200 μl of saline into the dorsal vein of the penis. To determine nonspecific binding of the radiopharmaceutical, a separate group of rats was injected s.c. with 0.5 mg of octreotide in 1 ml of 0.05 M acetic acid in saline, 30 min before injection of the radiolabeled peptide. At the indicated time points, rats were sacrificed under ether anesthesia. Organs and blood were collected, and the radioactivity in these samples was determined using a LKB-1282-Compugammasystem. Statistical evaluation was performed using one-way ANOVA, followed by comparison among class means and Student's *t* test, corrected for multiple pairwise comparisons between means.

RESULTS

Radiolabeling. ^{111}In-labeling efficiency of the different peptides and radioiodination efficiency of [Tyr³]octreotide ranged from 97 to 100%.

***In Vitro* Receptor Binding Studies.** Table 1 shows that unlabeled peptides had high and specific binding for the octreotide-binding receptors (mostly sst_2) on AtT20 membranes; the IC_{50} values were all in the nanomolar range. [DOTA⁰,Tyr³]octreotide showed the highest affinity.

***In Vitro* Internalization Studies.** Table 2 shows specific internalization, that is, total internalization corrected for internalization in the presence of a blocking dose of octreotide of the ^{111}In-labeled peptides in the octreotide receptor-positive rat pancreatic cell lines after a 60-min incubation at 37°C. Data were expressed as percentages of specific internalization of [^{125}I-Tyr³]octreotide. As is shown, internalized radioactivity of [^{111}In-DTPA⁰,Tyr³]octreotate was the highest of the compounds tested, whereas that of [^{111}In-DTPA⁰,D-Tyr¹]octreotide was the lowest.

Tissue Distribution in Rats. Fig. 2 presents radioactivity in octreotide binding receptor-positive (mostly sst_2) organs, including pancreas, adrenals, pituitary, and CA20948 rat pancreatic tumors, 4, 24, and 48 h after injection of the radiolabeled peptides. Uptake in these octreotide receptor-expressing organs at the time points tested was highest for [^{111}In-DTPA⁰,Tyr³]octreotate and lowest for [^{111}In-DTPA⁰,D-Tyr¹]octreotide.

Table 3 shows that uptake of ^{111}In-labeled peptides in these octreotide receptor-positive target organs represented mostly specific binding to the octreotide receptors because uptake was decreased to less than 7% of control by pretreatment of the rats with 0.5 mg of unlabeled octreotide, except for [^{111}In-DTPA⁰,D-Tyr¹]octreotide, for which uptake was decreased to about 30% of control.

In Table 4, radioactivity in octreotide receptor-negative organs and blood 24 h after injection of the tested ^{111}In-labeled peptides is shown. Clearance from the blood was rapid and comparable for all peptides. The radiolabeled peptides were excreted in the urine very rapidly and mostly intact; over 95% of the excreted radioactivity after 24 h was intact radiolabeled peptide (data not shown). Furthermore, the low uptake of [^{111}In-DTPA⁰,Tyr³]octreotate in the liver is worth mentioning, which is favorable, especially in combination with the rapid blood clearance and high uptake of this compound in the target organs.

The data obtained *in vivo*, as shown in Fig. 2 and Table 4, are summarized in Fig. 3, in which the AUC (h·%ID/g) for each group between 4 and 48 h post injection is shown. The top panel shows that radioactivity/g tissue in this time period was low in blood, liver, and spleen and higher in kidneys, tumor, and octreotide receptor (sst_2)-positive organs. The bottom panel shows the same data but expressed as percentage of the AUC of [^{111}In-DTPA⁰]octreotide in the different organs. In the sst_2-negative organs, AUC is comparable for the peptides, except for the low liver AUC of [^{111}In-DTPA⁰,Tyr³]octreotate. In the sst_2-positive organs, the AUC of [^{111}In-DTPA⁰,Tyr³]octreotate is the highest.

DISCUSSION

Compared to ^{123}I-Tyr³-octreotide, [^{111}In-DTPA⁰]octreotide was the preferred analogue for *in vivo* scintigraphy because it has several advantages: general availability, simple one-step method of radiolabeling, longer physiological half-life in plasma, and a more suitable metabolism (5). A new field of application of radiolabeled SS ana-

Table 2 *Comparison of specific internalization of [^{111}In-DTPA⁰]octreotide, [^{111}In-DTPA⁰,D-Tyr¹]octreotide, [^{111}In-DTPA⁰,Tyr³]octreotide, [^{111}In-DTPA⁰,Tyr³]octreotate, and [^{111}In-DOTA⁰,Tyr³]octreotide after a 60-min incubation at 37°C*

Data for each experiment expressed as percentage of specific [^{125}I-Tyr³]octreotide internalization (range, 6.5 ± 0.8%–9.2 ± 1.1% dose) tested in the same experiment. Data are the means of those obtained in at least two different experiments in the two octreotide receptor-positive cell lines used (CA20948 and AR42J).

Compound	Mean (SD)
[^{125}I-Tyr³]octreotide	100 (12)
[^{111}In-DTPA⁰]octreotide	8.2 (0.7)[a]
[^{111}In-DTPA⁰,D-Tyr¹]octreotide	2.2 (1.1)[a]
[^{111}In-DTPA⁰,Tyr³]octreotide	40.2 (4.5)[a]
[^{111}In-DTPA⁰,Tyr³]octreotate	211.5 (12)[a]
[^{111}In-DOTA⁰,Tyr³]octreotide	14.6 (1.1)[a]

[a] $P < 0.001$ *versus* [^{125}I-Tyr³]octreotide.

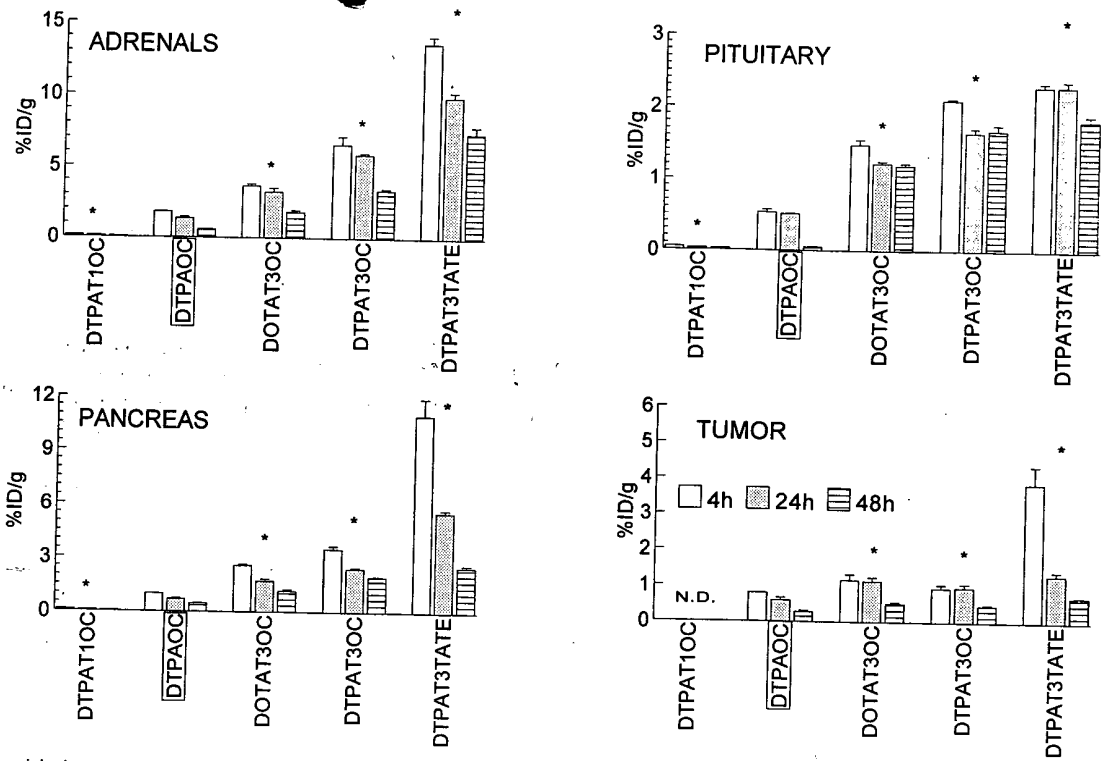

Fig. 2. Radioactivity in octreotide receptor-expressing organs 4, 24, and 48 h after injection of the ^{111}In-labeled peptides in rats. *Columns*, mean %ID/g ($n \geq 6$); *bars*, SE. *ND*, not determined; *DTPAOC* [DTPA^0]octreotide; *DTPAT1OC*, [DTPA^0,D-Tyr^1]octreotide; *DTPAT3OC*, [DTPA^0,Tyr^3]octreotide; *DTPAT3TATE*, [DTPA^0,Tyr^3]octreotate; *DOTAT3OC*, [DOTA^0,Tyr^3]octreotide. *, $P < 0.001$ *versus* [^{111}In-DTPA^0]octreotide, for all time points tested.

Table 3 *Radioactivity in SS receptor-positive organs of octreotide-pretreated rats 24 h after administration of the ^{111}In-labeled peptides*

Labeled compound was injected 30 min after s.c. injection of 0.5 mg of unlabeled octreotide or vehicle (control). Tissue radioactivity in octreotide-pretreated rats is expressed as a percentage of that in controls [for each group $n \geq 6$ mean (SE)].

Treatment	Pituitary	Pancreas	Adrenals	Tumor
[DTPA^{0}octreotide	6.9 (0.7)[a]	3.5 (0.03)[a]	1.5 (0.02)[a]	4.3 (0.3)[a]
[DTPA^0,D-Tyr^1]octreotide	22.2 (4.5)[a]	27.9 (1.4)[a]	29.6 (1.1)[a]	ND[b]
[DTPA^0,Tyr^3]octreotide	4.7 (0.4)[a]	0.9 (0.03)[a]	4.0 (0.3)[a]	4.8 (0.4)[a]
[DTPA^0,Tyr^3]octreotate	3.2 (0.4)[a]	2.6 (0.4)[a]	0.7 (0.1)[a]	6.3 (0.9)[a]
[DOTA^0,Tyr^3]octreotide	1.6 (0.04)[a]	1.0 (0.02)[a]	1.0 (0.04)[a]	3.6 (0.3)[a]

[a] $P < 0.001$ *versus* control.
[b] ND, not determined.

logues is the use of radiolabeled peptide for radionuclide therapy of receptor-positive lesions. Currently, this application was explored successfully by repeated administration of high doses of [^{111}In-DTPA^0]octreotide in humans (8). However, a β^- particle emitter, such as ^{90}Y, may, in certain cases, appear more suitable for this purpose than the Auger electron emitter ^{111}In. Radiotherapeutic use of ^{90}Y-labeled peptide will lead to a higher and more evenly distributed radiation dose to the tumor because of its larger particle range and tissue penetration. Even tumors with an nonhomogeneous cellular distribution of receptors, such as breast tumors, may respond favorably to treatment with such a ^{90}Y-labeled radiopharmaceutical, whereas treatment with ^{111}In-labeled peptide will not be successful because of the particle range of the Auger electrons, which is only about one cell diameter. Because ^{90}Y-DTPA is unstable, introduction of the DOTA chelator was necessary, enabling stable radiolabeling with ^{90}Y and ^{111}In.

We investigated receptor binding, internalization and biodistribution characteristics of several SS analogues, all labeled with ^{111}In: [DTPA^0]-octreotide, [DTPA^0,Tyr^3]octreotide, [DTPA^0,D-Tyr^1]octreotide, [DOTA^0,Tyr^3]octreotide, and [DTPA^0,Tyr^3]-octreotate. Phe residues were replaced with Tyr to increase the hydrophilicity of the peptides. Furthermore, [DTPA^0,Tyr^3]octreotate, with the C-terminal threonine, was synthesized to investigate the effects of an additional negative charge on clearance and cellular uptake.

For the success of radionuclide therapy, it is important that the radiopharmaceutical is internalized by the tumor cells after binding to the receptor. Internalization of [^{111}In-DTPA^0]octreotide into human neuroendocrine tumor cells was described recently (15). Here, we also observed specific internalization of the tested ^{111}In-labeled peptides. Internalized radioactivity of all radiolabeled peptides was higher than that of [^{111}In-DTPA^0]octreotide, except that of [^{111}In-DTPA^0,D-Tyr^1]octreotide.

The results of the *in vitro* binding studies demonstrated that all unlabeled peptides showed high and specific binding for the octreotide receptors. *In vivo*, uptake of the ^{111}In-labeled peptides in octreotide receptor-expressing tissues was also demonstrated to be highly specific. Our findings further showed that specific uptakes of ^{111}In-labeled [DTPA^0,Tyr^3]octreotide, [DOTA^0,Tyr^3]octreotide, and [DTPA^0,Tyr^3]octreotate in octreotide receptor-expressing tissues were significantly higher than that of [^{111}In-DTPA^0]octreotide at the

Table 4 *Radioactivity in SS receptor-negative organs and blood of rats 24 h after administration of the ^{111}In-labeled peptides*

Tissue radioactivity is expressed as %ID/g [for each group $n \geq 6$, mean (SE)].

Treatment	Blood	Liver	Kidney	Spleen
[DTPA^0]octreotide	0.003 (0.000)	0.05 (0.003)	1.91 (0.11)	0.03 (0.002)
[DTPA^0,D-Tyr^1]octreotide	0.003 (0.001)	0.03 (0.001)[a]	1.52 (0.05)[a]	0.03 (0.002)
[DTPA^0,Tyr^3]octreotide	0.003 (0.001)	0.06 (0.004)	1.39 (0.08)[a]	0.03 (0.002)
[DTPA^0,Tyr^3]octreotate	0.003 (0.000)	0.02 (0.001)[a]	1.96 (0.11)	0.02 (0.001)
[DOTA^0,Tyr^3]octreotide	0.002 (0.001)	0.05 (0.000)	2.32 (0.13)[a]	0.04 (0.001)

[a] $P < 0.01$ *versus* [^{111}In-DTPA^0]octreotide.

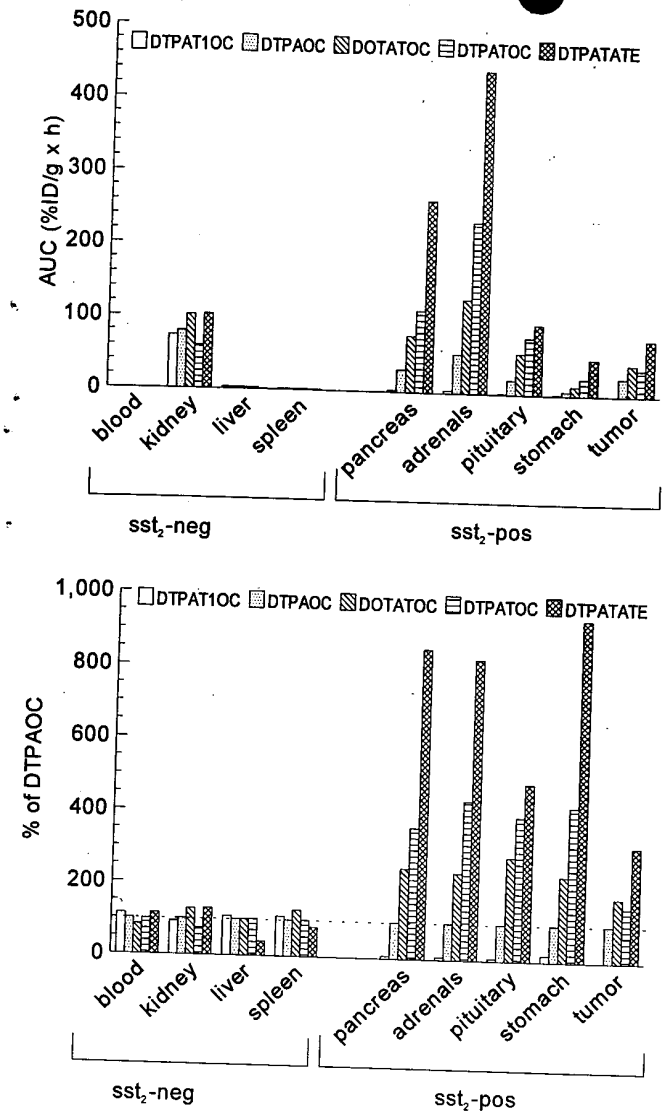

Fig. 3. *Top*, AUC of radioactivity between 4 and 48 h after injection of the different ^{111}In-labeled peptides in rats. *Columns*, mean h·%ID/g. *DTPAOC*, [DTPA^0]octreotide; *DTPAT1OC*, [DTPA^0,D-Tyr^1]octreotide; *DTPAT3OC*, [DTPA^0,Tyr^3]octreotide; *DTPAT3TATE*, [DTPA^0,Tyr^3]octreotate; *DOTAT3OC*, [DOTA^0,Tyr^3]octreotide. *Bottom*, same as *top*, except data represent percentage of [DTPA^0]octreotide AUC (*columns*).

time points tested. Uptake of [^{111}In-DTPA^0,D-Tyr^1]octreotide was significantly lower, in accordance with the lower internalization rate found *in vitro*. In our *in vivo* animal model, [^{111}In-DTPA^0,Tyr^3]octreotate showed the highest uptake in the octreotide receptor-positive organs and tumor of the ^{111}In-labeled peptides tested, also in accordance with the *in vitro* internalization studies. Because blood radioactivity was comparable for all radiolabeled peptides, we also found that [^{111}In-DTPA^0,Tyr^3]octreotate had the highest tumor-to-blood ratio. Uptake of [DTPA^0,Tyr^3]octreotide was higher than that of [DOTA^0,Tyr^3]octreotide, both *in vitro* and *in vivo*, in the octreotide receptor-positive organs; however, uptake in the target, the tumor, was not significantly different for these two radiolabeled peptides; therefore, the therapeutic index of [Tyr^3]octreotide has not been impaired significantly by the replacement of DTPA for DOTA, necessary for ^{90}Y studies (11). We are currently investigating the relationship of the injected peptide mass and uptake in target organs for these two peptides to further elucidate the consequences of the DTPA-to-DOTA replacement.

At radiotherapeutic levels, the high uptake of radioactivity in the octreotide receptor-positive normal organs, such as adrenals and pituitary, should also be considered. We are performing radionuclide therapy studies in normal rats with [DOTA^0,Tyr^3]octreotide radiolabeled with different radionuclides to investigate possible radiotoxic effects on normal organs. However, until now, no radiotoxicity was found in these organs.

The ^{111}In-labeled peptides are rapidly cleared from the body, mostly by the kidneys. However, a significant amount of the dose accumulated in the kidneys, reducing both the scintigraphic sensitivity for detection of small tumors in the perirenal region in the abdomen and the application for radionuclide therapy. It has been reported that renal accumulation of peptides or proteins labeled with radiometals can be reduced by both L- and D-lysine (16–19). Recently, we described that D-lysine administration resulted in a significant reduction of labeled [DTPA^0]octreotide, [DTPA^0,Tyr^3]octreotide, and [DOTA^0,Tyr^3]octreotide uptake in the kidneys without affecting uptake in receptor-positive tissues, which is favorable for both visualization of lesions in the kidney region and for radionuclide therapy, thus bringing these applications further within reach (10).

It can be concluded that ^{111}In-labeled [DTPA^0,Tyr^3]octreotide and, especially, [DTPA^0,Tyr^3]octreotate and their DOTA-coupled counterparts are most promising for scintigraphy and, after coupling to therapeutic radionuclides, for radionuclide therapy of octreotide receptor-positive tumors in humans.

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Cancer Research

AN OFFICIAL JOURNAL OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

AACR Annual Meeting Advance Registration and Housing Forms Inside

February 1, 1998
Volume 58 • Number 3
PP. 377–580
ISSN 0008-5472 • CNREA 8

Articles

Comparison of Four ^{64}Cu-Labeled Somatostatin Analogues in Vitro and in a Tumor-Bearing Rat Model: Evaluation of New Derivatives for Positron Emission Tomography Imaging and Targeted Radiotherapy[‖]

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Received October 27, 1998

Previous studies have shown that modification of the somatostatin analogue octreotide (OC), by substitution of tyrosine for phenylalanine at position 3 and of a C-terminal carboxylic acid for an alcohol, to give Tyr^3-octreotate (Y3-TATE) improved uptake of the peptide in somatostatin receptor-positive tissues. To determine which substitution best accounts for increased target tissue uptake, the peptides containing single modifications, Tyr^3-octreotide (Y3-OC) and octreotate (TATE), were synthesized. These peptides were conjugated to the macrocyclic chelating agent 1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid (TETA) and radiolabeled with ^{64}Cu(II). The in vitro receptor binding, in vitro tumor cell uptake, and in vivo distribution properties of ^{64}Cu-labeled TETA-Y3-OC and TETA-TATE were compared to those of [^{64}Cu]TETA-OC and [^{64}Cu]TETA-Y3-TATE. Cu-TETA-TATE (IC_{50} = 0.297 ± 0.0055 nM) and Cu-TETA-Y3-TATE (IC_{50} = 0.308 ± 0.0375 nM) displayed significantly higher binding affinity to somatostatin receptors on CA20948 rat pancreatic tumor membranes than Cu-TETA-Y3-OC (IC_{50} = 0.397 ± 0.0206 nM) and Cu-TETA-OC (IC_{50} = 0.498 ± 0.039 nM). Similarly, the uptakes of [^{64}Cu]TETA-Y3-TATE (60.75 ± 1.21%) and [^{64}Cu]TETA-TATE (55.62 ± 0.16%) into AR42J rat pancreatic tumor cells over a 2-h time period were higher than those of [^{64}Cu]-TETA-Y3-OC (47.20 ± 1.20%) and [^{64}Cu]TETA-OC (34.07 ± 2.24%). The in vitro results suggest that the C-terminal carboxylate may contribute more to enhanced receptor binding and tumor cell uptake than the substitution at the 3-position. Biodistributions in CA20948 tumor-bearing rats showed receptor-mediated uptake of the ^{64}Cu-labeled peptides in somatostatin-rich tissues, including the pituitary, adrenals, pancreas, and tumor. The structure−activity relationships of the four ^{64}Cu-labeled peptides did not show consistent trends in all target tissues, but [^{64}-Cu]TETA-Y3-TATE exhibited tumor uptake 1.75−3.5 times higher than the other derivatives at 4 h postinjection. The greater tumor retention of [^{64}Cu]TETA-Y3-TATE justifies the selection of this agent for future PET imaging and targeted radiotherapy studies.

Introduction

The targeting of somatostatin receptors with radiolabeled peptides has led to the development of agents for both diagnostic imaging and radiotherapy of cancer. Octreotide (OC), an 8-amino acid analogue of somatostatin, has been radiolabeled and used to image somatostatin receptor-positive tumors in humans by positron emission tomography (PET) and single photon emission computed tomography (SPECT). For these purposes, somatostatin analogues have been labeled with a number of β^+- and γ-emitting radionuclides, including ^{111}In, ^{123}I, ^{99m}Tc, ^{68}Ga, ^{64}Cu, ^{18}F, and ^{86}Y.[1-8] In the United States and Europe, ^{111}In-DTPA-OC (In-111 Pentetreotide) is approved for routine clinical use in the diagnosis of neuroendocrine cancer. In addition, widespread interest in targeted radiotherapy has led to the labeling of somatostatin analogues with a variety of cytotoxic radionuclides. For example, [^{161}Tb]DTPA-OC,[9] [^{90}Y]DTPA-OC,[10] [^{188}Re]RC-160,[11] [^{90}Y]DOTA-Tyr^3-OC,[12,13] [^{64}Cu]TETA-OC,[14] and [^{64}Cu]TETA-Tyr^3-TATE[15] are being evaluated for radiotherapeutic efficacy in animal models and clinical trials.

Copper-64 ($t_{1/2}$ = 12.7 h, β^+ = 0.655 MeV (19.3%), β^- = 0.573 MeV (39.6%)) is an attractive radionuclide for both PET imaging and radiotherapy. Large quantities of high-specific activity ^{64}Cu can be produced on demand using a biomedical cyclotron.[16] The applications of ^{64}Cu for PET imaging and targeted radiotherapy through attachment to biologically active molecules have been reviewed.[17] The first ^{64}Cu-labeled somatostatin ana-

[‖] Abbreviations: DTPA, diethylenetriaminepentaacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; TETA, 1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid; Y3, tyrosine-3; OC, octreotide; TATE, octreotate; MALDI FTMS, matrix-assisted laser desorption−ionization Fourier transform mass spectrometry.

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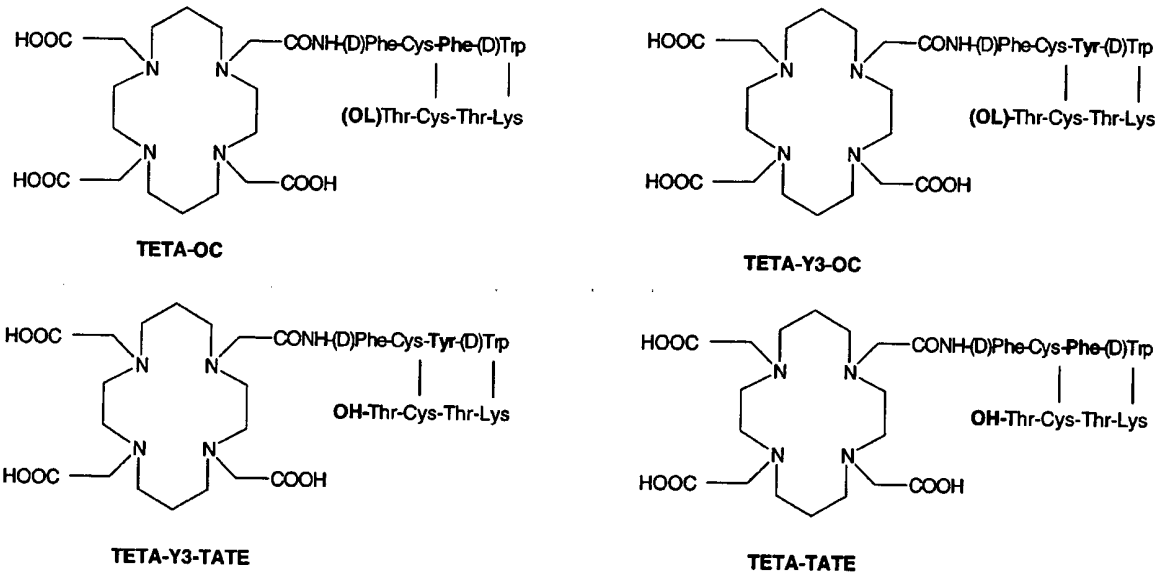

Figure 1. Structures of TETA-OC, TETA-Y3-OC, TETA-Y3-TATE, and TETA-TATE.

logue, [^{64}Cu]TETA-D-Phe^1-octreotide ([^{64}Cu]TETA-OC), displayed high-affinity somatostatin receptor binding in vitro and in vivo.[7] Evaluation of this agent in eight neuroendocrine cancer patients showed that PET imaging with [^{64}Cu]TETA-OC detected more lesions than γ-scintigraphy using [^{111}In]DTPA-OC.[18] Subsequent evaluation of the therapeutic efficacy of [^{64}Cu]TETA-OC has demonstrated growth inhibition of somatostatin receptor-positive tumors in rats at doses exhibiting minimal toxicity.[14]

Studies have shown that subtle modification of the octreotide peptide leads to improved uptake in receptor-rich tissues. The substitutions of a tyrosine (Y) for phenylalanine (F) in the 3-position and of a C-terminal carboxylic acid for an alcohol improved uptake of the peptide in adrenals, pancreas, pituitary, and tumor.[19-21] These findings were confirmed by our own studies, where [^{64}Cu]TETA-D-Phe^1-Tyr^3-octreotate ([^{64}Cu]TETA-Y3-TATE) demonstrated significantly greater uptake than [^{64}Cu]TETA-OC in the somatostatin-rich tissues of two tumor-bearing animal models.[22]

In the current investigation, we examined the effects of single modifications to the octreotide peptide on target tissue uptake, to determine which alteration best accounts for the improvements observed with [^{64}Cu]TETA-Y3-TATE. The substitution of the tyrosine for phenylalanine in the 3-position afforded the peptide Tyr^3-octreotide (Y3-OC), and changing the C-terminus from an alcohol to a carboxylic acid produced the analogue Phe^3-octreotate (TATE). Both of these peptides were subsequently conjugated to TETA (1,4,8,11-tetraazacyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) and radiolabeled with ^{64}Cu. We studied receptor-mediated uptake of these two peptides in vitro and in a tumor-bearing animal model and compared the results to those obtained with ^{64}Cu-labeled TETA-OC and TETA-Y3-TATE.

Results

Synthesis and Radiolabeling of Peptides. OC, Y3-OC, TATE, and Y3-TATE (Figure 1) were synthesized by the solid-phase Fmoc method and conjugated with the 1-hydroxybenzotriazole ester of tri-*tert*-butyl TETA on the resin. It should be noted that while these peptides have what is termed TETA conjugated to them, the TETA in use is actually a monoamide derivative of TETA wherein one of the carboxylates has been used to form an amide bond with the peptide. After reversed-phase HPLC, all peptide conjugates were isolated in 90–96% purity. The exact masses of the peptides were confirmed by high-resolution MALDI FTMS, which showed errors of 0.2–6 ppm between observed and calculated values. The ^{64}Cu-labeled peptides were obtained in >98% radiochemical purity, as determined by radio thin-layer chromatography (radio-TLC), in specific activities ranging from 0.5 to 2.5 mCi/μg (18.5–92.5 MBq/μg).

Receptor Binding Assays. The displacement of [^{64}Cu]TETA-OC by the natural copper complexes of TETA-OC, TETA-Y3-TATE, TETA-Y3-OC, and TETA-TATE on rat CA20948 pancreatic tumor cell membranes is shown by the curves presented in Figure 2. All four unlabeled conjugates bound specifically to somatostatin receptors with high affinities. IC_{50} values were 0.308 ± 0.0375 nM for Cu-TETA-Y3-TATE, 0.397 ± 0.0206 nM for Cu-TETA-Y3-OC, and 0.297 ± 0.0055 nM for Cu-TETA-TATE. The value for Cu-TETA-OC, was previously reported to be 0.498 ± 0.039 nM.[14]

AR42J Cell Uptake Studies. The uptakes of ^{64}Cu-labeled TETA-Y3-TATE, TETA-Y3-OC, TETA-OC and TETA-TATE into AR42J rat pancreatic tumor cells during a 2-h incubation at 37 °C are shown in Figure 3. The somatostatin receptor density (B_{max}) on AR42J cells was previously determined by our group to be 148.8 fmol/mg of protein.[23] Thus, under the conditions employed, cell uptake was measured at a 10-fold molar excess of somatostatin receptor to peptide. At 15 min, accumulation of [^{64}Cu]TETA-OC in AR42J cells was 10.23 ± 2.38% of the total activity administered, with uptake increasing to 34.07 ± 2.24% at 2 h. Uptakes of ^{64}Cu-labeled TETA-Y3-OC and TETA-TATE were similar at 15 min (22.77 ± 2.38% and 20.36 ± 1.89%,

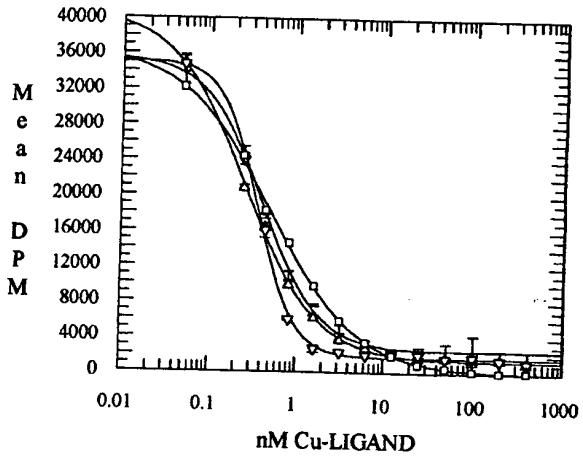

Figure 2. Displacement curves of [^{64}Cu]TETA-OC from CA20948 rat pancreatic tumor cell membranes. Results represent the mean of quadruplicate measurements using ^{nat}Cu-TETA-OC (□), ^{nat}Cu-TETA-Y3-TATE (△), ^{nat}Cu-TETA-Y3-OC (—), or ^{nat}Cu-TETA-TATE (▽).

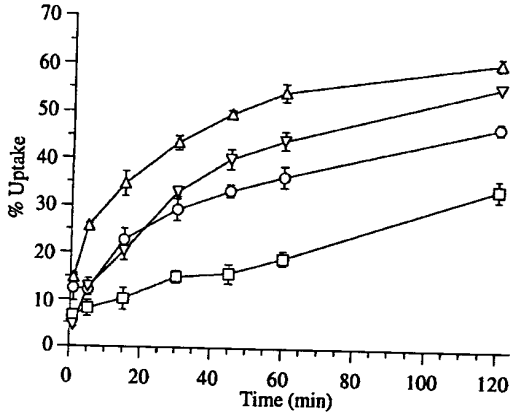

Figure 3. Percentage uptake of [^{64}Cu]TETA-OC (□), [^{64}Cu]-TETA-Y3-TATE (△), [^{64}Cu]TETA-Y3-OC (○), and [^{64}Cu]TETA-TATE (▽) into AR42J cells over time.

respectively) but differed significantly at 2 h (47.20 ± 1.20% and 55.62 ± 0.16%, respectively). Both [^{64}Cu]-TETA-Y3-OC and [^{64}Cu]TETA-TATE exhibited significantly greater accumulation in AR42J cells than [^{64}Cu]-TETA-OC at all time points. Uptake of [^{64}Cu]TETA-Y3-TATE was 34.68 ± 2.53% after 15 min and continued to increase to 60.75 ± 1.21% at 2 h. Over the 2-h experimental period, [^{64}Cu]TETA-Y3-TATE showed the greatest accumulation of the four analogues in AR42J cells. Compared to the other derivatives, the increased uptake of [^{64}Cu]TETA-Y3-TATE was statistically significant at all time points, with the exception of [^{64}Cu]-TETA-TATE at 2 h.

Animal Biodistribution Studies. The uptakes of [^{64}Cu]TETA-Y3-OC and [^{64}Cu]TETA-TATE in pancreas, adrenals, liver, and tumor are shown in Figure 4. For comparison, previously published biodistribution data for [^{64}Cu]TETA-Y3-TATE[22] and [^{64}Cu]TETA-OC[7] are also presented in Figure 4. The results represent biodistributions performed with a similar mass of each radiolabeled peptide (5–8 ng). The results of blocking experiments, using either Y3-TATE or OC to compete with the receptor-mediated uptake of [^{64}Cu]TETA-Y3-TATE, are shown in Figure 5.

Both [^{64}Cu]TETA-Y3-OC and [^{64}Cu]TETA-TATE displayed rapid blood clearance after 1 h. The nontarget organs, e.g., kidney, brain, and liver, showed similar uptake for all four peptide conjugates, with no significant differences. The receptor-rich tissues (adrenals, pancreas, pituitary, and tumor) did not show any significant difference in uptakes between [^{64}Cu]TETA-Y3-OC and [^{64}Cu]TETA-TATE (adrenals, 8.01 ± 1.61% ID/g vs 5.93 ± 1.20% ID/g; pancreas, 4.45 ± 0.96% ID/g vs 5.13 ± 0.92% ID/g; pituitary, 3.41 ± 0.76% ID/g vs 3.69 ± 0.80% ID/g; tumor, 2.17 ± 0.66% ID/g vs 1.76 ± 1.15% ID/g, respectively).

[^{64}Cu]TETA-Y3-TATE had higher uptake in all receptor-rich tissues (except adrenals) than did the other analogues at 1 h (adrenals, 9.07 ± 1.24% ID/g; pancreas, 9.35 ± 1.66% ID/g; pituitary, 6.47 ± 1.77% ID/g; tumor, 2.37 ± 0.44% ID/g) ($p < 0.001$). The trend of adrenal uptakes revealed that ^{64}Cu-labeled TETA-Y3-OC and TETA-Y3-TATE had higher accumulation at 1 and 4 h postinjection than the corresponding Phe^3 analogues. With the exception of the tumor, [^{64}Cu]TETA-Y3-TATE, [^{64}Cu]TETA-Y3-OC, and [^{64}Cu]TETA-TATE all demostrated at least 2-fold higher uptake than [^{64}Cu]TETA-OC in receptor-positive organs. At 1 h, tumor uptakes of [^{64}Cu]TETA-Y3-OC and [^{64}Cu]TETA-TATE were similar to the values obtained with [^{64}Cu]TETA-Y3-TATE and [^{64}Cu]TETA-OC. However, at 4 h, the tumor uptake of [^{64}Cu]TETA-Y3-TATE (2.22 ± 0.26% ID/g) was significantly higher than that of [^{64}Cu]TETA-Y3-OC (1.28 ± 0.25% ID/g) and [^{64}Cu]TETA-TATE (0.63 ± 0.52% ID/g), as well as the tumor uptake of [^{64}Cu]TETA-OC at 3 h (0.63 ± 0.05% ID/g).

In the ligand competition experiments, more than 90% of the uptake of [^{64}Cu]TETA-Y3-TATE in somatostatin-rich tissues was blocked with a co-injection of either unlabeled Y3-TATE or unlabeled OC. At 1 h, co-injection of Y3-TATE decreased the pancreatic uptake of [^{64}Cu]TETA-Y3-TATE significantly more than co-injection of OC (0.15 ± 0.02% ID/g vs 0.76 ± 0.13% ID/g, respectively) ($p < 0.005$). The same trend is seen in the adrenals (0.17 ± 0.02% ID/g for Y3-TATE and 0.26 ± 0.09% ID/g for OC) and the tumor (0.22 ± 0.02% ID/g for Y3-TATE and 0.64 ± 0.10% ID/g for OC) at 1 h postinjection. Interestingly, the bone also shows receptor-mediated uptake of [^{64}Cu]TETA-Y3-TATE. Using Y3-TATE as the blocking agent, bone uptake was decreased from 0.61 ± 0.08% ID/g to 0.09 ± 0.02% ID/g at 1 h; a blocking dose of OC decreased the bone uptake to 0.13 ± 0.02% ID/g at the same time point. Co-injection with blocking peptides did not have a significant effect on uptake in nontarget organs.

Discussion

[^{64}Cu]TETA-OC is currently being investigated for clinical PET imaging of neuroendocrine cancer.[18] Preliminary results with this compound are encouraging in that more tumors have been visualized with this agent than with [^{111}In]DTPA-OC. [^{64}Cu]TETA-OC has also been evaluated for targeted radiotherapy in a tumor-bearing rat model.[14] However, it suffers from the disadvantages of less than optimal blood clearance and rapid tumor clearance. On the basis of previous results obtained with ^{111}In-labeled octreotide analogues,[20,21] we have evaluated [^{64}Cu]TETA-Y3-TATE in vitro and in

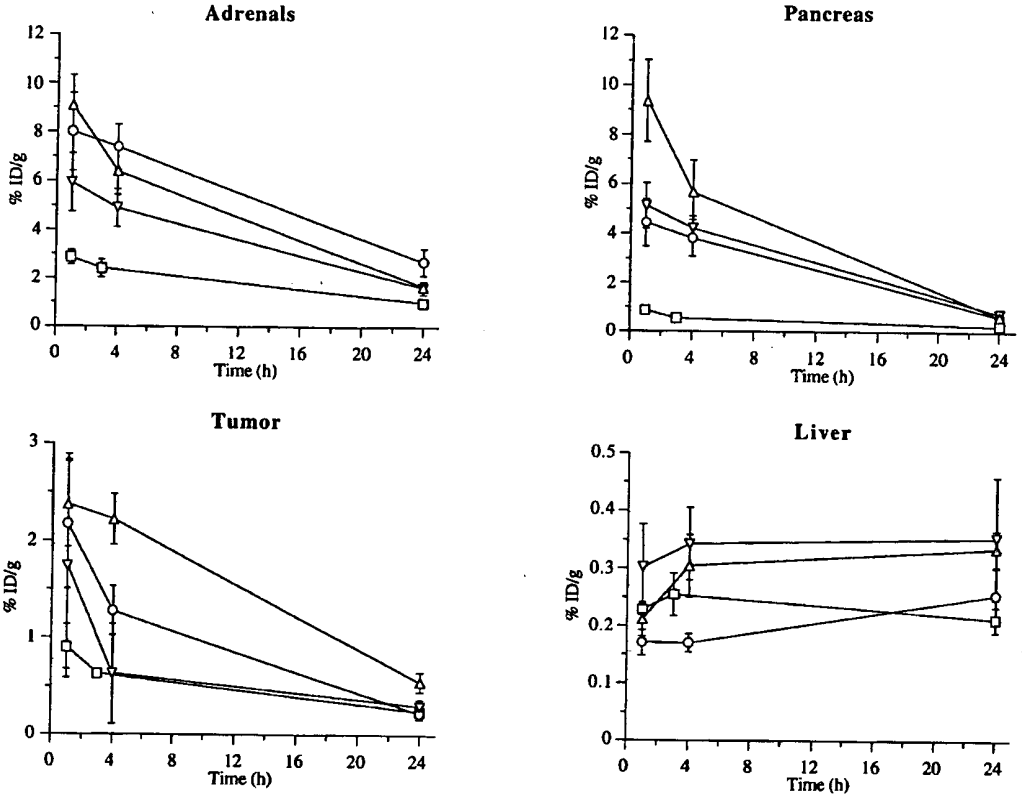

Figure 4. Uptake in selected organs of [^{64}Cu]TETA-OC (□), [^{64}Cu]TETA-Y3-TATE (△), [^{64}Cu]TETA-Y3-OC (○), and [^{64}Cu]TETA-TATE (▽) in Lewis rats bearing CA20948 rat pancreatic tumors. Standard deviations (SD) are indicated; all data were corrected for radiodecay. Note differences in *y*-axis scales.

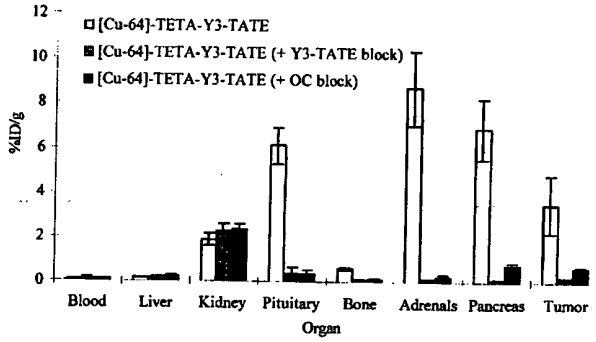

Figure 5. Biodistributions at 1 h of [^{64}Cu]TETA-Y3-TATE, [^{64}Cu]TETA-Y3-TATE co-injected with 150 μg of Y3-TATE, and [^{64}Cu]TETA-Y3-TATE co-injected with 150 μg of OC in Lewis rats bearing CA20948 rat pancreatic tumors. Standard deviations (SD) are indicated; all data were corrected for radiodecay.

two animal models as a potential agent for PET imaging[22] and targeted radiotherapy.[15] [^{64}Cu]TETA-Y3-TATE demonstrated rapid blood clearance in CA20948-bearing Lewis rats, with tumor uptake twice that of [^{64}Cu]TETA-OC. Moreover, tumor:blood ratios were over 4-fold higher at 1 h for [^{64}Cu]TETA-Y3-TATE.

[^{64}Cu]TETA-Y3-TATE differs from the parent compound, [^{64}Cu]TETA-OC, by the substitutions of tyrosine for phenylalanine in the 3-position and a C-terminal carboxylic acid for an alcohol. The current study was undertaken to determine how these modifications contribute to the increase in uptake of [^{64}Cu]TETA-Y3-TATE in receptor-rich tissues. Two peptides, TETA-TATE and TETA-Y3-OC, were synthesized, radiolabeled with ^{64}Cu, and evaluated in Lewis rats bearing CA20948 pancreatic tumors. Compared to the parent peptide OC, TETA-Y3-OC contains the substitution of tyrosine in the 3-position, while TETA-TATE incorporates the change in C-terminus from an alcohol to an acid.

In vitro receptor binding studies showed that all peptides evaluated bound specifically to somatostatin receptors on CA20948 membranes with high relative affinities. The parent compound, Cu-TETA-OC, had the lowest affintity for the receptor, while Cu-TETA-Y3-TATE and Cu-TETA-TATE had the highest affinities. Cu-TETA-Y3-OC exhibited a lower affinity for the receptor than the TATE derivatives, but its IC_{50} value was still significantly lower than that of Cu-TETA-OC. These results suggest that the C-terminal modification may contribute more to high-affinity receptor binding than the substitution at position 3.

The AR42J rat pancreatic carcinoma cell line is also known to express somatostatin receptors both in vitro and in vivo.[24,25] To evaluate and compare the cellular uptake of the radiolabeled peptides in vitro, the AR42J cell line was utilized. Under the conditions employed, the mass of each peptide added was identical, and the somatostatin receptor concentration was 10-fold greater than the peptide concentration. Therefore the results obtained are a direct comparison of the accumulation rates of the analogues and likely represent a combination of membrane binding, internalization, and cellular retention of the compounds. The data revealed that [^{64}Cu]TETA-Y3-TATE had the highest uptake in AR42J cells, followed by [^{64}Cu]TETA-TATE, [^{64}Cu]TETA-Y3-

OC, and [^{64}Cu]TETA-OC in descending order. As in the case of the receptor binding studies, these results showed that the C-terminal carboxyl modification makes a greater contribution to increased cell uptake than the substitution at position 3.

The results of the cell uptake studies are in agreement with the findings of de Jong et al.,[20,26] who reported that the amounts of ^{111}In-labeled somatostatin analogues internalized into CA20948 and AR42J cells followed the trend DTPA-Y3-TATE > DTPA-Y3-OC > DOTA-Y3-OC > DTPA-OC. While those studies distinguished between internalized and membrane-bound ligand, they did not include DTPA- or DOTA-TATE derivatives, so the individual contributions of the C-terminal and 3-position modifications could not be assessed. The studies by de Jong et al. were conducted at peptide concentrations as low as 100 pM. The results described here were obtained at peptide concentrations of exactly 30 pM to give a receptor:ligand molar ratio of 10:1. Receptor excess is desirable for comparison of cellular uptakes because it mimics the physiological conditions of tumor targeting in vivo.

The rat biodistribution studies clearly demonstrated that the uptakes of [^{64}Cu]TETA-Y3-TATE, [^{64}Cu]TETA-Y3-OC, and [^{64}Cu]TETA-TATE in receptor-positive normal tissues are significantly higher at 1 and 4 h than that of [^{64}Cu]TETA-OC. The tyrosine-substituted analogues, [^{64}Cu]TETA-Y3-TATE and [^{64}Cu]TETA-Y3-OC, showed higher uptake in the adrenals than the corresponding phenylalanine-substituted derivatives. This finding suggests that the presence of the tyrosine residue may be responsible for increased adrenal uptake, possibly a result of the increased hydrophilicities of these peptides. In the pancreas and pituitary, [^{64}Cu]-TETA-Y3-TATE showed the highest uptakes at 1 h, while [^{64}Cu]TETA-TATE and [^{64}Cu]TETA-Y3-OC had similar intermediate uptakes, and [^{64}Cu]TETA-OC exhibited much lower uptakes than the other three analogues. In these target tissues, the combination of C-terminal and residue 3 modifications may have a synergistic effect on uptake. These observations are consistent with the findings of de Jong et al.,[20,21] who showed increased target tissue uptake with ^{111}In-labeled DTPA-Y3-OC and DTPA-Y3-TATE derivatives.

The in vivo ligand competition experiments demonstrated that uptake of [^{64}Cu]TETA-Y3-TATE is receptor-mediated in all target tissues. Moreover, Y3-TATE was generally more effective as a blocking agent than OC, a finding which may be attributable to its higher affinity for somatostatin receptors or differences in internalization rates or uptake kinetics. The same ligand competition effect was also observed in bone, suggesting that bone uptake of [^{64}Cu]TETA-Y3-TATE was also receptor-mediated.

Tumor uptakes of the four ^{64}Cu-labeled octreotide analogues at 1 h were more similar than the uptakes in other target tissues. At this time point, [^{64}Cu]TETA-OC had the lowest tumor uptake. While [^{64}Cu]TETA-Y3-TATE had the highest accumulation in tumor at 1 h, this value was not significantly different than those obtained with ^{64}Cu-labeled TETA-TATE and TETA-Y3-OC. However, at 4 h postinjection, tumor uptake of [^{64}Cu]TETA-Y3-TATE was 1.75–3.5 times higher than those of the other analogues. The longer residence time of [^{64}Cu]TETA-Y3-TATE in the tumor may increase its efficacy for targeted radiotherapy and justify future therapy studies using this agent.

It is evident from these investigations that modification of the 3-position amino acid and alteration of the C-terminus both contribute to increased target tissue uptake of ^{64}Cu-labeled octreotide analogues. While the structure–activity relationships of these four analogues do not show consistent uptake trends in all target tissues that identify the superior compound, the greater accumulation and retention of [^{64}Cu]TETA-Y3-TATE in tumor provide a rationale to select this agent for future targeted radiotherapy studies. We are continuing to evaluate the therapeutic efficacy of [^{64}Cu]TETA-Y3-TATE in the CA20948 rat model in preparation for clinical trials.

Experimental Section

Materials. ^{64}Cu was produced on a biomedical cyclotron at Washington University School of Medicine by previously reported methods.[16] All chemicals, unless otherwise stated, were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). All solutions were prepared using ultrapure water (18 MΩ-cm resistivity). Thin-layer chromatography was performed using Whatman MKC$_{18}$F reversed-phase TLC plates with 10% ammonium acetate:methanol (30:70) as the mobile phase. Radio-TLC detection was accomplished using a BIOSCAN System 200 imaging scanner (Washington, DC). Radioactive samples were counted on a Beckman 8000 γ counter (Irvine, CA). Adult male Lewis rats (230–290 g) were purchased from Harlan Sprague–Dawley, Inc. (Indianapolis, IN). The rat pancreatic tumor CA20948[27] was obtained from the Tumor Bank at Biomeasure, Inc. (Hopkinton, MA) and was maintained by serial passage in animals.

Peptide Synthesis. Solid-phase peptide synthesis (SPPS) was performed on an Applied Biosystems model 432A "synergy" peptide synthesizer employing the Fmoc (9-fluorenylmethoxycarbonyl) method. Instrument protocol required 25 μmol of subsequent Fmoc-protected amino acids activated by a combination of 1-hydroxybenzotriazole (HOBt) and 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). The Fmoc-protected amino acids were purchased commercially unless otherwise stated; the prepacked amino acids were obtained from Perkin-Elmer (Norwalk, CT), while those unavailable in prepacked form, such as the D-amino acids and Fmoc-Cys(Acm), were supplied by BACHEM Bioscience, Inc. (King of Prussia, PA) or Novabiochem (San Diego, CA). Tri-*tert*-butyl TETA was synthesized internally by a modification of the published procedure.[28] Exact mass measurements on the peptide conjugates were performed by Mass Consortium (San Diego, CA), using an IonSpec Fourier transform ion cyclotron mass spectrometer with a 4.7-T superconducting magnet. Samples in 2,5-dihydroxybenzoic acid matrix were irradiated with a nitrogen laser (LaserScience, Inc.) operated at 337 nm.

The synthesis of TETA-Y3-TATE, TETA-OC, TETA-Y3-OC, and TETA-TATE was accomplished by previously reported methods.[22] The peptide conjugates were purified by reversed-phase HPLC, using a Vydac Protein & Peptide C$_{18}$ column (2.2 × 25 cm) and a linear gradient from 10% to 70% solvent B (solvent A, 0.1% TFA; solvent B, 0.1% TFA/90% CH_3CN) over 40 min at a flow rate of 10 mL/min. Detection was accomplished at 230 nm. Pure fractions were identified by analytical HPLC using two diverse systems: system A, HPLC on a Vydac diphenyl (219TP54) column (0.46 × 25 cm) and a linear gradient from 2% to 98% solvent B (solvent A, 0.1% TFA; solvent B, 0.1% TFA/CH_3CN) over 100 min at a flow rate of 1 mL/min, with detection at 214 and 280 nm; system B, reversed-phase HPLC on a Vydac Protein & Peptide C$_{18}$ column (0.46 × 25 cm), with detection at 214 nm. For TETA-Y3-TATE, TETA-Y3-OC, and TETA-TATE, analytical reversed-phase HPLC was performed using a solvent gradient starting with

0% solvent B for 2 min, followed by a linear gradient from 0% to 70% solvent B (solvent A, 0.1% TFA/5% CH_3CN; solvent B, 0.1% TFA/90% CH_3CN) over 15 min at a flow rate of 0.5 mL/min. For TETA-OC, analytical reversed-phase HPLC was carried out using a linear gradient from 5% to 70% solvent B (solvent A, 0.1% TFA; solvent B, 0.1% TFA/90% CH_3CN) over 15 min at a flow rate of 2 mL/min. The peptides were also analyzed by high-resolution MALDI FTMS. TETA-Y3-TATE: HPLC retention times = 33.0 min (system A), 11.2 min (system B); MALDI FTMS *m/z* calcd for $C_{67}H_{95}N_{14}O_{19}S_2$ $(M + H)^+$ = 1463.6339, found 1463.6343. TETA-OC: HPLC retention times = 35.9 min (system A), 10.8 min (system B); MALDI FTMS *m/z* calcd for $C_{67}H_{97}N_{14}O_{17}S_2$ $(M + H)^+$ = 1433.6598, found 1433.6609. TETA-Y3-OC: HPLC retention times = 32.5 min (system A), 11.1 min (system B); MALDI FTMS *m/z* calcd for $C_{67}H_{97}N_{14}O_{18}S_2$ $(M + H)^+$ = 1449.6547, found 1449.6646. TETA-TATE: HPLC retention times = 36.3 min (system A), 12.3 min (system B); MALDI FTMS *m/z* calcd for $C_{67}H_{95}N_{14}O_{18}S_2$ $(M + H)^+$ = 1447.6390, found 1447.6404.

Radiolabeling of Peptide Conjugates. The conjugated peptides were labeled with ^{64}Cu(II) according to previously reported methods for the preparation of [^{64}Cu]TETA-OC[7] and [^{64}Cu]TETA-Y3-TATE.[22] Briefly, 1−5 mCi (37−185 MBq) of ^{64}Cu in 0.1 M ammonium acetate, pH 5.5, was added to 1−10 μg of the peptide conjugate in 0.1 M ammonium acetate, pH 5.5. Gentisic acid (1 mg/mL) was added to the labeling mixture to counteract the effects of radiolysis. The solution was incubated for 1 h at room temperature. The radiolabeled peptide was purified on a C-18 SepPak cartridge, using 100% ethanol as the elution solvent, and radiochemical purity was determined by radio-TLC.

Receptor Binding Assays. The receptor binding assays were performed using [^{64}Cu]TETA-OC on membranes obtained from CA20948 tumors harvested from euthanized rats. The competing ligands, ^{nat}Cu-TETA-OC, ^{nat}Cu-TETA-Y3-TATE, ^{nat}Cu-TETA-Y3-OC, and ^{nat}Cu-TETA-TATE, were prepared by the reaction of high-purity natural copper acetate, using the same procedure described above for preparation of the ^{64}Cu-labeled peptides. Purity of the final products were confirmed by HPLC, using the same method described for purification of the TETA conjugates. IC_{50} values were determined according to previously published methods,[14] using the Millipore MultiScreen assay system (Bedford, MA). Data analysis was performed using the programs GraFit (Erithacus Software, U.K.), LIGAND (NIH, Bethesda, MD), and GraphPad PRISM (San Diego, CA). Each data point represents the mean of four experimental values.

Cell Uptake Studies. The apparatus and procedures for the cell uptake experiments are based on previously described methods.[29,30] Briefly, the AR42J cell line was maintained by serial passage in monolayers in Dulbecco's modified Eagle's media (DMEM), supplemented with 10% fetal bovine serum, in a humidified 5% CO_2 atmosphere at 37 °C. Viability of the cells and cell numbers were measured by trypan blue exclusion procedures using a hemacytometer. The cell viability before and after the experiments was determined to be >95% in all cases. Cells were harvested from monolayers with cell dissociation solution (Sigma Chemical Co., St. Louis, MO) and resuspended in fresh DMEM media at a concentration of 2×10^6 cells/mL. An aliqout of 0.3 pmol of the radiolabeled peptide (1.11 μCi of [^{64}Cu]TETA-OC, 2.13 μCi of [^{64}Cu]TETA-Y3-TATE, 1.94 μCi of [^{64}Cu]TETA-Y3-OC, or 1.93 μCi of [^{64}Cu]TETA-TATE) was added to 10 mL of cells, which were incubated at 37 °C with continuous agitation. At 1, 5, 15, 30, 45, 60, and 120 min triplicate 200-μL aliquots were removed and placed in ice. The cells were immediately isolated by centrifugation, and the percent uptake of the compound into the cells was calculated as described.[30]

Animal Biodistribution Studies. Using a 21G Trocar, the somatostatin receptor-positive rat pancreatic tumor CA20948 (1-mm^3 piece) was implanted subcutaneously into the nape of the neck of male Lewis rats (230−290 g). The tumors were allowed to grow for 10 days, until approximately 4 g in size. The ^{64}Cu-labeled peptide conjugate (5.4 μCi, 5 ng) was injected intravenously via the tail vein into CA20948 tumor-bearing Lewis rats. Animals were euthanized at 1, 4, and 24 h postinjection. The tumor, blood, lung, liver, spleen, kidney, muscle, fat, heart, brain, pituitary, bone, adrenals, pancreas, stomach, small intestine, upper large intestine, and lower large intestine were removed, drained of blood, weighed, and counted in a γ counter. By comparison with a standard representing the injected dose per animal, the samples were corrected for radioactive decay, to calculate percent injected dose per gram (% ID/g) of tissue and percent injected dose per organ (% ID/organ).

Ligand Competition Experiments. [^{64}Cu]TETA-Y3-TATE (5.4 μCi, 5 ng) was injected intravenously via the tail vein into CA20948-bearing Lewis rats. Two additional groups of animals were co-injected with [^{64}Cu]TETA-Y3-TATE (5.4 μCi, 5 ng) and either 150 μg of unlabeled Y3-TATE or 150 μg of unlabeled OC. All three groups of animals were sacrificed at 1 h postinjection, after which biodistributions were obtained as described above.

Statistical Methods. To compare differences between the ^{64}Cu-labeled peptides, a Student's *t*-test was performed. Differences at the 95% confidence level ($p < 0.05$) were considered significant.

Acknowledgment. The authors wish to thank Dr. Deborah W. McCarthy and Todd A. Perkins for production of ^{64}Cu, as well as Elizabeth L. C. Sherman, Margaret M. Morris, and Lynne A. Jones for their excellent technical assistance. We also wish to thank Randy Wilhelm for help in analysis of the high-resolution mass spectral data and W. Barry Edwards for help with the HPLC analysis. This work was supported by NIH Grant CA64475 and Mallinckrodt, Inc.

Supporting Information Available: Tables of mean percent injected dose per gram (% ID/g) of [^{64}Cu]TETA-Y3-OC, [^{64}Cu]TETA-TATE, and [^{64}Cu]TETA-Y3-TATE with two blocking agents and percent injected dose per organ (% ID/organ) with standard deviations for 13 tissues and 3 time points evaluated and also synthesis of mono-*N*-(carboxymethyl)-tris-*N,N,N*-(*tert*-butyloxycarbonylmethyl)cyclam (tri-*tert*-butyl TETA) from cyclam. This information is available free of charge via the Internet at http://pubs.acs.org.

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JM980602H

Radiotherapy and Dosimetry of ^{64}Cu-TETA-Tyr^3-Octreotate in a Somatostatin Receptor-positive, Tumor-bearing Rat Model[1]

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ABSTRACT

^{64}Cu [$T_{1/2}$ = 12.8 h; β^+ = 0.655 MeV (19%); β^- = 0.573 MeV (40%)] has shown promise as a radioisotope for targeted radiotherapy. It has been demonstrated previously that the somatostatin analogue ^{64}Cu-TETA-octreotide (^{64}Cu-TETA-OC, where TETA is 1,4,8,11-tetraazacyclotetradecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid) significantly inhibited the growth of somatostatin receptor-positive CA20948 rat pancreatic tumors in Lewis rats (C. J. Anderson *et al.*, J. Nucl. Med., *39:* 1944–1951, 1998). In this study, we evaluated the radiotherapeutic efficacy of a new ^{64}Cu-labeled somatostatin analogue, ^{64}Cu-TETA-Tyr^3-octreotate (^{64}Cu-TETA-Y3-TATE), in CA20948 tumor-bearing rats. A single dose of 15 mCi (555 MBq) of ^{64}Cu-TETA-Y3-TATE was shown to be more effective in reducing tumor burden than the same dose of ^{64}Cu-TETA-OC. In multiple dose experiments, tumor-bearing rats were administered three doses of either 10 or 20 mCi (370 or 740 MBq) of ^{64}Cu-TETA-Y3-TATE at 48-h intervals. Rats given 3 × 10 mCi (3 × 370 MBq) showed extended mean survival times compared with rats given a single dose; however, no complete regressions occurred. Complete regression of tumors was observed for all rats treated with 3 × 20 mCi (3 × 740 MBq), with no palpable tumors for ~10 days; moreover, the mean survival time of these rats was nearly twice that of controls. Toxicity was determined by physical appearance and hematological and enzyme analysis, which revealed no overt toxicity and only transient changes in blood and liver chemistry. Absorbed dose estimates showed the dose-limiting organ to be the kidneys. The radiotherapy results, along with absorbed dose estimates to target and clearance organs, confirm that ^{64}Cu-labeled somatostatin analogues warrant continued consideration as agents for targeted radiotherapy.

INTRODUCTION

Somatostatin analogues have been investigated for utility in scintigraphic and PET[3] imaging of cancer in humans. For example, ^{111}In-pentetreotide (^{111}In-DTPA-octreotide; Refs. 1 and 2) has been approved for routine clinical use in the diagnosis of neuroendocrine cancer in the United States and Europe. Somatostatin analogues have also been labeled with other radionuclides and evaluated as possible radiotherapeutic agents. Targeted radiotherapy studies have been performed in animal models with somatostatin analogues labeled with ^{90}Y (3–5), ^{111}In (6), and ^{64}Cu (7) with varying degrees of success, and of these agents, ^{111}In-DTPA-octreotide (8–10) and ^{90}Y-DOTA-Tyr^3-octreotide (^{90}Y-SMT 487, ^{90}Y-DOTATOC, or ^{90}Y-DOTA-Y3-OC; Ref. 11) are being investigated in ongoing clinical radiotherapy trials.

Improvement in target tissue uptake of radiolabeled somatostatin analogues has been the focus of a number of studies. It has been shown that substitution of a tyrosine (Y) for phenylalanine (F) in the 3-position and changing the C-terminus from an alcohol to a carboxylic acid increases uptake of the peptide in receptor-rich tissues (12–14). This has been confirmed by our own studies, where ^{64}Cu-TETA-Tyr^3-octreotate (^{64}Cu-TETA-Y3-TATE; Fig. 1) demonstrated significantly greater uptake in somatostatin-rich tissues in two tumor-bearing animal models (Lewis rats bearing CA20948 tumors and severe combined immunodeficient mice bearing AR42J tumors) compared with ^{64}Cu-TETA-octreotide (^{64}Cu-TETA-OC; Fig. 1; Refs. 15 and 16).

^{64}Cu [$T_{1/2}$ = 12.8 h; β^+ = 0.655 MeV (19%); β^- = 0.573 MeV (40%)] has diverse applications in radiopharmaceutical chemistry for PET imaging (17) as well as therapy (7, 18). Moreover, ^{64}Cu can be produced on demand in high yield and in high specific activity on a small biomedical cyclotron (19), making it a radionuclide available to many medical institutions.

We report herein an investigation into the radiotherapeutic potential of ^{64}Cu-TETA-Y3-TATE in CA20948 tumor-bearing

Received 1/12/99; revised 7/20/99; accepted 7/20/99.
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[1] This work was supported by Grant CA64475 from the NIH (to C. J. A.), a grant from Mallinckrodt, Inc. (to C. J. A.), and Grant 59200 from the United States Department of Energy (to P. D. C.).
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[3] The abbreviations used are: PET, positron emission tomography; Y3, tyrosine-3; TETA, 1,4,8,11-tetraazacyclotetradecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; DOTA, 1,4,7,10-tetraazacyclododecane-*N*,*N'*,*N''*,*N'''*-tetraacetic acid; OC, octreotide; TATE, octreotate; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ID/g, injected dose per gram; MIRD, medical internal radiation dose; ROI, region of interest.

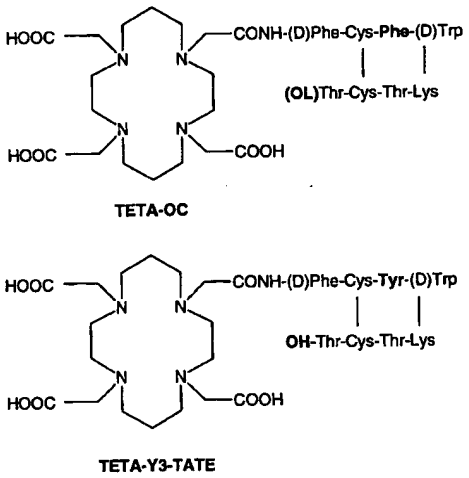

Fig. 1 Structures of TETA-OC (*top*) and TETA-Y3-TATE (*bottom*).

rats, a model of somatostatin receptor-positive pancreatic cancer. The therapeutic efficacy of ^{64}Cu-TETA-Y3-TATE was compared with ^{64}Cu-TETA-OC and control agents. In addition, hematological, liver, and kidney assays were performed to evaluate the potential toxicity of the ^{64}Cu-TETA-Y3-TATE. Human absorbed dose estimates for ^{64}Cu-TETA-Y3-TATE were calculated from both rat biodistribution data and PET imaging of a baboon.

MATERIALS AND METHODS

Synthesis of ^{64}Cu-TETA-OC and ^{64}Cu-TETA-Y3-TATE. ^{64}Cu was produced on a biomedical cyclotron at the Washington University School of Medicine by methods reported previously (19). ^{64}Cu-TETA-OC and ^{64}Cu-TETA-Y3-TATE were prepared according to literature methods (15, 16, 20), in specific activities ranging from 1.25 to 2.5 mCi/μg (46–93 MBq/μg).

Animal Models. All animal experiments were conducted in compliance with the Guidelines for the Care and Use of Research Animals established by Washington University's Animal Studies Committee. The rat pancreatic tumor CA20948 (21) was obtained from the Tumor Bank at Biomeasure, Inc. (Hopkinton, MA). Adult male Lewis rats (230–290 g) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The CA20948 cell line was maintained by serial passage in animals. In rat experiments, male Lewis rats were injected with a 1-mm^3 tumor section of CA20948 tumor 10 days prior to treatment as described previously (7).

Radiotherapy Experiments (Single Dose). Tumor-bearing rats (tumor volume, 0.3–1.0 cm^3) were injected with one dose of 200 μl of 0.1 M NH_4OAc (buffer), 15 μg of TETA-Y3-TATE, 15.5 mCi (574 MBq) of ^{64}Cu-TETA-OC, or 15.5 mCi (574 MBq) of ^{64}Cu-TETA-Y3-TATE. The tumor volume was measured every 1–3 days (using calipers), and rats were sacrificed by administration of Metofane, followed by cervical dislocation, when the tumors reached a volume of ~10 cm^3 or became ulcerated.

Radiotherapy Experiments (Multiple Dose). Two different multiple dose protocol experiments were carried out. In the first experiment, one group of tumor-bearing rats (tumor volume, 0.3–1.5 cm^3) received three 10-mCi (370 MBq) doses of ^{64}Cu-TETA-Y3-TATE, with a control group receiving equivalent masses of unlabeled TETA-Y3-TATE. Rats were injected 10, 12, and 14 days after implantation of tumor cells. The second experiment was identical, except that the treated group of rats received three 20-mCi (740 MBq) doses of ^{64}Cu-TETA-Y3-TATE 10, 12, and 14 days after implantation, and the control group received equivalent masses of unlabeled TETA-Y3-TATE on those days. Tumor volumes were measured, and animals were sacrificed in a manner identical to that described for the single-dose radiotherapy experiments.

Blood Chemistry. Hematological, liver, and kidney chemistries were studied for the group of tumor-bearing rats that received 3 × 20 mCi. These results were compared with those obtained from rats treated with control agents. In each group, anesthetized rats were weighed, and blood was removed by cardiac puncture during the posttherapy survival period. Toxicity analysis was performed by the Diagnostic Services Laboratory in the Department of Comparative Medicine at Washington University School of Medicine. The hematology analysis included WBC counts, RBC counts, platelet counts, as well as measurement of hemoglobin, hematocrit, and differential WBCs. Liver and kidney analysis included blood urea nitrogen, creatinine, ALP, ALT, and AST.

Effect of Specific Activity on Biodistribution of Radiolabeled Peptide. To determine the effect of peptide mass on the biodistribution of ^{64}Cu-TETA-Y3-TATE, groups of CA20948 tumor-bearing rats (n = 5) were coinjected with 10 ng of ^{64}Cu-TETA-Y3-TATE (5 μCi, 0.2 MBq) and a known mass of TETA-Y3-TATE to give final injectates of 10, 50, 100, 500, 1000, and 5000 ng. All animals were sacrificed at 1 h, and % ID/g and % ID/organ values of selected tissues and organs were determined.

Rat Dosimetry. The estimated human absorbed doses of ^{64}Cu-TETA-Y3-TATE to normal organs were obtained using biodistribution data in CA20948 tumor-bearing rats, according to methods described previously (7). ^{64}Cu-TETA-Y3-TATE (35 μCi, 1.3 MBq) was injected i.v., and the rats were euthanized by Metofane overdose and cervical dislocation at 1, 3, 6, 12, 24, 36, and 48 h after injection. The rats for the 48-h time point were housed in metabolism cages to determine % ID excreted in urine and feces at 1, 3, 6, 17, 24, 43, and 48 h. Time-activity curves were generated for 12 organs. Cumulative activity (μCi-h or kBq-h) was determined by numerically integrating the area under the time-activity curves. Human dose estimates were then calculated using standard MIRD techniques, and S-values (mean absorbed dose per unit cumulative activity) for ^{64}Cu were obtained from the MIRDOSE3 program (22). Bone activity was assumed to be distributed equally between the trabecular bone and cortical bone. The absorbed dose to the CA20948 rat tumor was determined from biodistribution data using methods described previously (18). Briefly, time-activity data were determined by combining the tumor data from different time points after injection. Each tumor was excised and weighed, and an S-value was calculated for the average tumor size for each time point, assuming a spherical tumor of unit density. Nonpenetrat-

ing emissions of ^{64}Cu were assumed to be completely absorbed in the tumor. For penetrating emissions, appropriate specific absorbed fractions for the 511 and 1340 keV photons of ^{64}Cu were used. This approach assumes that tumors of similar size in different animals demonstrate similar uptake characteristics, and the resulting absorbed dose is an average of that absorbed by each tumor.

Baboon Dosimetry. The biodistribution of ^{64}Cu-TETA-Y3-TATE was also determined in a 25-kg male baboon by PET imaging. PET imaging was performed using a Siemens/CTI ECAT EXACT PET system (CTI PET Systems, Knoxville, TN) to determine the biodistribution of 4.6 mCi (170.2 MBq) of ^{64}Cu-TETA-Y3-TATE over the first 18 h after injection. Images of the animal's torso were acquired at 30-min intervals from 0 to 3 h and then again at 18 h after injection. The baboon was anesthetized for the first 3 h and then repositioned to approximately the same position the following day.

Activity concentration values were derived from the PET images, which were calibrated previously against the same dose calibrator (Capintec, Ramsey, NJ) used to assay the injected dose. Corrections for photon attenuation, random coincidences, deadtime, and scatter were applied. Images were reconstructed with filtered backprojection and modest smoothing (Hann, cutoff 0.3 pixels^{-1}), so that most organs could be clearly identified. ROIs were drawn over liver, spleen, kidneys, bladder, blood pool, red marrow, and muscular soft tissue and were used to estimate total organ accumulations of the compound. Blood activity was taken to be the average of the maximum pixels in five to six adjacent slices through the left ventricle, which was necessary to avoid partial volume effects in the moving heart. Liver activity was taken as the average value in a large ROI centered in the liver and averaged > five to six slices. Kidney activity, although trapped primarily in the renal cortex, was taken as the average value inside a ROI outlining the entire kidney and was assumed to be uniformly distributed in the organ. ROI values were decay-corrected to the time of injection, extrapolated where necessary by standard human organ and blood volumes, and then normalized to the baboon's weight (23). By comparison with the total injected activity, the % ID to each organ was determined. Bone marrow activity was derived from blood pool activity according to the model of Siegel *et al.* (24) using a partition fraction of 0.3.

Time-activity curves were generated from the PET ROI results for the seven organs. Each was fit with a biexponential function (Fig. 2) and then integrated numerically to determine the residence time of the activity in each organ. The results accounted for 90–99% of the injected activity over the imaging period, with the rest being included as "missing" and assigned to the MIRD category "remainder-of-body." Thus, the missing fraction was assumed to be distributed uniformly in the body. The "residence time," or accumulation-time product, can be determined for each relevant organ by integrating the time-activity curve either analytically, from 0 to ∞, or numerically over a suitably long interval. We used a numerical integration from 0 to 48 h. The absorbed radiation dose to a given organ was calculated as the sum of the products of the residence times and the tabulated S-values for ^{64}Cu in a standard human geometry. Because the bladder residence time depends primarily on the pattern of voiding, we have calculated a conservative estimate assuming no excretion, as well as a more realistic estimate using the dynamic bladder model available in the MIRDOSE3 software package, with a voiding interval of 4 h.

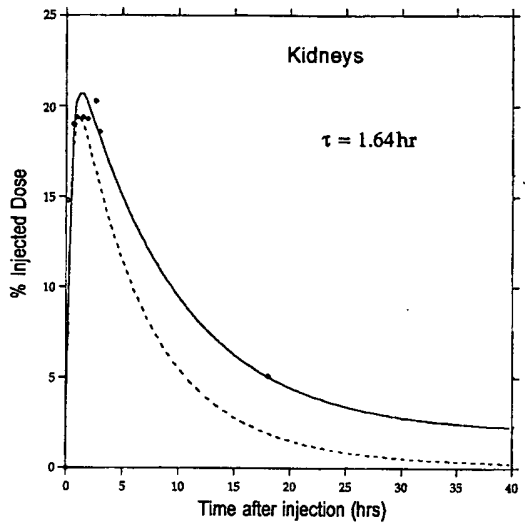

Fig. 2 Time-activity curves for kidneys (*A*) and blood (*B*) from PET images of a baboon injected with ^{64}Cu-TETA-Y3-TATE. Data points in the *upper curve* (*solid line*) are corrected for physical decay, forming the basis for the biexponential fit. The *lower curve* is the fitted curve with physical decay included, from which the residence time, τ, is derived by numerical integration.

Statistical Analysis. To determine statistical significance in the biodistribution studies, a Tukey's Studentized Range (HSD) Test was performed with $P < 0.05$ being consid-

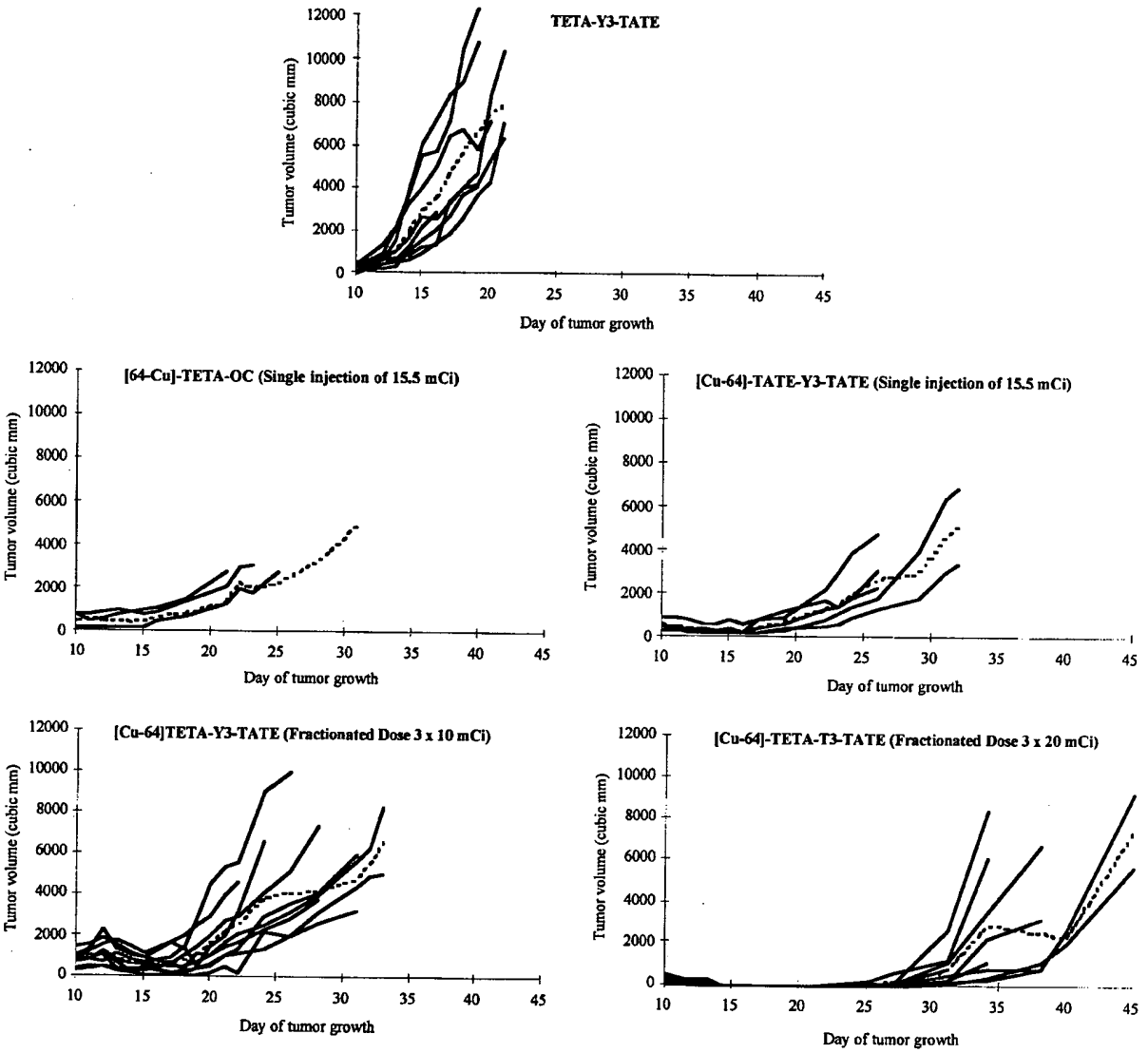

Fig. 3 Radiotherapy experiments in CA20948 tumor-bearing rats. Comparison of tumor growth in control rats and rats treated with ^{64}Cu-TETA-OC and ^{64}Cu-TETA-Y3-TATE. ———, individual rats; ----, mean values.

ered significantly different. In the therapy studies, Tukey's Studentized Range (HSD) Test, ANOVA, Scheffe's test, and a Least Difference Test were performed to compare single-dose and multiple-dose protocols and to compare these with data obtained from control rats. Comparison was made on the length of time it took the tumor to reach 10,000 mm^3 in size or the time to ulceration.

RESULTS

Radiotherapy Experiments (Single Dose). The results from all of the radiotherapy studies undertaken in CA20948 tumor-bearing rats are shown in Fig. 3. All rats that received a single 15.5-mCi dose of ^{64}Cu-TETA-Y3-TATE showed significant reduction in tumor volume (29–73%) over the following 7-day period, taking 28.4 ± 3.29 days for the tumor volume to reach >10,000 mm^3 or to ulcerate after tumor implant. Inhibition of tumor growth and reduction of tumor size after a single dose of ^{64}Cu-TETA-Y3-TATE were more apparent ($P < 0.05$) than that observed in animals receiving an equal dose of ^{64}Cu-TETA-OC (0–35% reduction over 7 days). There was no tumor growth inhibition in the control groups, and the time it took for the tumors to reach >10,000 mm^3 or to ulcerate (20.71 ± 2.06 days for TETA-Y3-TATE and 19.44 ± 1.67 days for buffer) were significantly lower than the groups that received a single dose of ^{64}Cu-TETA-Y3-TATE ($P < 0.05$). The single 15.5-mCi dose of ^{64}Cu-TETA-Y3-TATE reduced and inhibited the growth of the CA20948 tumor for over 10 days before normal growth resumed. There was no weight loss or appearance of

toxicity with ^{64}Cu-TETA-Y3-TATE at the 15.5-mCi dose. It is important to note that tumors that regrew after remission often ulcerated at an earlier stage than those in controls groups.

Radiotherapy Experiments (Multiple Dose). In both multiple dose experiments, the control groups (10–15 μg of unlabeled TETA-Y3-TATE) showed unrestricted growth of the tumor (mean survival time, 19.25 ± 2.63 days). All rats receiving the 3 × 10 mCi dose regimen of ^{64}Cu-TETA-Y3-TATE showed tumor growth inhibition and a decrease in tumor volume of 36–81%. The smallest tumor sizes were recorded between days 16 and 18 after implantation (0.07–0.60 cm^3). In the treated rats, it took an average of 28.44 ± 3.91 days for the tumors to reach >10,000 mm^3 or to ulcerate after implant. In rats receiving a 3 × 20 mCi dose regimen, there were no palpable tumors in any of the animals at day 14 after tumor implantation. Complete remission and disappearance of the tumor was observed for 10 days. On day 25 after the implant, tumors began to reappear and continued to grow more slowly than before the therapy, until the animals had to be sacrificed 38.22 ± 4.27 days after tumor implant. By using the Tukey's Studentized Range Test, we found that the time it took the tumors to reach >10,000 mm^3 in size or to ulcerate in the rats receiving a 3 × 20 mCi dose regimen was significantly higher than all other groups examined in all other therapy and control protocols ($P < 0.05$).

Blood Chemistry and Physical Appearance. Toxicity was determined in rats receiving 3 × 20 mCi of ^{64}Cu-TETA-Y3-TATE by monitoring weight and gross physical appearance, as well as hematological and liver and kidney function. The mean weight of the treated rats increased similarly to that of the control rats, and they maintained a healthy physical appearance (with no sign of scruffy coat or diarrhea) over the experimental period. Blood chemistries were compared with baseline levels obtained from the control rats. The mean WBC count decreased to 25–50% of the control group level at day 12 after the tumor implant (5,500 ± 3,630/mm^3 *versus* 13,600 ± 1,170/mm^3). The transient drop in WBCs remained constant until day 15 (7,500 ± 1,690/mm^3) and then was seen to recover to baseline levels by day 34 (9,530 ± 2,290/mm^3) after the tumor implant. The differential WBCs showed a transient elevation in segregated neutrophils to a maximum of 407% of controls after 17 days (61.0 ± 5.29% *versus* 15.0 ± 1.63%), with a transient decrease in lymphocytes to a minimum of 45% of controls at day 17 (37.0 ± 4.55% *versus* 82.0 ± 2.16%). Both segregated neutrophils and lymphocyte levels were seen to recover to baseline levels by day 34 (23.75 ± 9.03% and 71.8 ± 8.66%, respectively). No significant changes in RBCs or hemoglobin or hematocrit levels were noted. Platelet levels initially elevated to 138% of controls up to day 17 (11,27.50 ± 106.59 × 10^3/mm^3 *versus* 815.25 ± 87.43 × 10^3/mm^3) and then decreased dramatically to 35% of controls by day 25 (282.25 ± 119.69 × 10^3/mm^3). Baseline platelet levels were reached by day 34 (969.00 ± 151.39 × 10^3/mm^3). The levels of kidney enzymes, blood urea nitrogen and creatinine, did not change significantly over the observation period. Levels of ALP, ALT, and AST were all seen to decrease (65.1, 51.9, and 49.9% of controls, respectively) by day 17. Both ALP and AST recovered to >75% of baseline levels by day 20, and ALP, ALT and AST all returned to baseline levels by day 34.

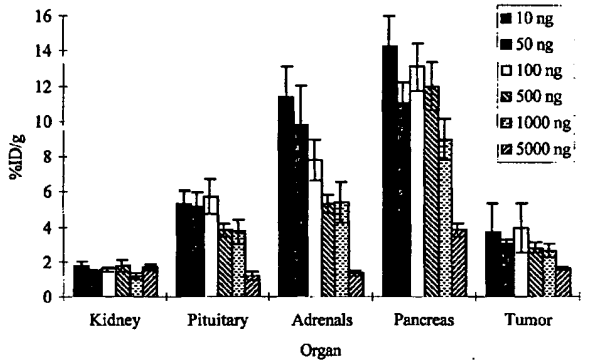

Fig. 4 Biodistribution at 1 h after injection of 5 μCi (0.2 MBq) of ^{64}Cu-TETA-Y3-TATE in CA20948-bearing rats at doses of 10, 50, 100, 500, 1000, and 5000 ng of peptide. *Bars,* SD.

Effect of Specific Activity on Biodistribution of Radiolabeled Peptide. The uptake of 5 μCi (0.2 MBq) of ^{64}Cu-TETA-Y3-TATE, diluted with different masses of unlabeled TETA-Y3-TATE, in receptor-rich organs and the kidney at 1 h after injection is shown in Fig. 4. With a coinjection of 5000 ng of peptide, uptake decreased significantly in receptor-positive tissues ($P < 0.05$). The uptake in the pituitary showed a 4-fold decrease (5.40 ± 0.74% ID/g for 10 ng *versus* 1.24 ± 0.24% ID/g for 5000 ng; $P < 0.05$), the adrenals displayed an 8-fold decrease (11.41 ± 01.67% ID/g for 10 ng *versus* 1.40 ± 0.19% ID/for 5000 ng; $P < 0.05$), the uptake in the pancreas was nearly 4-fold lower (14.24 ± 1.74% ID/g for 10 ng *versus* 3.84 ± 0.41% ID/g for 5000 ng; $P < 0.05$), and the tumor uptake was over 2-fold lower (3.69 ± 1.68% ID/g for 10 ng *versus* 1.64 ± 0.06% ID/g for 5000 ng; $P < 0.05$) at the highest mass injected. Other nontarget organs showed no significant decreases in uptake.

Dosimetry. Human absorbed dose estimates to normal organs, calculated from rat biodistribution data and baboon PET imaging, are shown in Table 1. The absorbed dose to the kidneys for ^{64}Cu-TETA-Y3-TATE, based on rat biodistribution, was 0.445 rad/mCi (0.120 mGy/MBq). By comparison, baboon PET image data showed a peak of 20% ID in the kidneys, with fairly rapid clearance, giving an absorbed dose of 1.25 rad/mCi (0.337 mGy/MBq). The intestinal tract also showed distinctly different uptake results from the rat and baboon experiments. There was virtually no uptake in the bowel of the baboon, and only a small fraction (0.9% ID) cleared via the hepatobiliary system. Table 1 also shows the effect of the dynamic bladder model on the dose to the urinary bladder wall in the PET imaging study. In the CA20948 rat model, the average absorbed dose to the tumor was calculated to be 40.8 rad/mCi (11.0 mGy/MBq) for a single injection of ^{64}Cu-TETA-Y3-TATE.

DISCUSSION

^{64}Cu-TETA-OC is presently being evaluated clinically at Washington University School of Medicine for the detection of neuroendocrine cancer by PET (25) and was investigated for therapeutic potential in a rodent tumor model (7). ^{64}Cu-TETA-Y3-TATE has been shown to have a higher affinity for the

Table 1 Absorbed radiation doses resulting from administration of ^{64}Cu-TETA-Y3-TATE, determined from rat biodistribution and PET imaging of a baboon

Organ	Rat rad/mCi (mGy/MBq)	Baboon rad/mCi (mGy/MBq)
Kidneys	0.445 (0.120)	1.25 (0.337)
Liver	0.108 (0.029)	0.39 (0.107)
Gallbladder		0.49 (0.132)
Red marrow	0.069 (0.018)	0.038 (0.009)
Spleen	0.053 (0.014)	0.35 (0.095)
Pancreas	0.145 (0.039)	0.16 (0.043)
Adrenals	0.616 (0.166)	0.059 (0.016)
Upper large intestine wall	0.244 (0.066)	0.035 (0.010)
Small intestine	0.131 (0.035)	0.044 (0.012)
Lower large intestine wall	1.030 (0.278)	0.059 (0.016)
Urinary bladder	0.785 (0.212)	2.82 (0.763)[a]/0.38 (0.103)[b]
Total body	0.040 (0.011)	0.063 (0.017)

[a,b] Urinary bladder dose calculated assuming: [a] no excretion; and [b] using the dynamic bladder model of Cloutier *et al.* (37).

somatostatin receptor than ^{64}Cu-TETA-OC both *in vitro* and *in vivo*; *in vitro*, IC_{50}s for the binding of Cu-TETA-Y3-TATE and Cu-TETA-OC to CA20948 pancreatic tumor cell membranes were 0.250 ± 0.05 nM and 0.498 ± 0.039 nM, respectively; *in vivo*, this increased affinity for somatostatin receptors was shown by a 2-fold uptake of ^{64}Cu-TETA-Y3-TATE over ^{64}Cu-TETA-OC into CA20948 tumors over 1 h (15). The aim of the present study was to determine the radiotherapeutic efficacy of the superior analogue, ^{64}Cu-TETA-Y3-TATE, in a tumor-bearing rodent model, to evaluate the toxicity of the agent, and to calculate human absorbed doses from both rodent biodistribution and primate imaging. The results obtained in this investigation strongly suggest that ^{64}Cu-TETA-Y3-TATE may be superior to ^{64}Cu-TETA-OC and has potential for targeted radiotherapy of neuroendocrine cancer in humans.

From the single-dose radiotherapy experiment (1 × 15.5 mCi), it is evident that ^{64}Cu-TETA-Y3-TATE is at least as effective as ^{64}Cu-TETA-OC in effecting greater tumor regression and may possibly be more effective. In the first multiple dose protocol using ^{64}Cu-TETA-Y3-TATE (3 × 10 mCi), the tumor burden decreased dramatically with an extended time for the tumor burden to reach >10,000 mm^3 or ulcerate in the treated animals compared with the control groups. In the second multiple dose regimen (3 × 20 mCi), there were no palpable tumors in the treated group for an extended period of time (~10 days). Moreover, the time for the tumor burden to reach >10,000 mm^3 or to ulcerate in these rats was nearly twice that of the control groups ($P < 0.05$). All of the statistical analysis performed confirmed that the survival time of the rodents was dependent on the dose administered. It was shown that the 3 × 20 mCi multiple dose regimen was more effective in tumor regression than single-dose administration and the first multiple dose protocol (3 × 10 mCi). The advantages of multiple dose protocols over a single dose have precedence in radioimmunotherapy studies (26, 27) and radiotherapy with peptides (7, 28) and include significant reduction of the tumor burden with decreased toxicity. A multiple dose regimen also has the advantage of delivering a consistent amount of tolerable radiation over an extended period to the tumor, while allowing intermittent recovery of nontarget tissues. The decreased toxicity is often attributable to decreased bone marrow suppression, which is the result of delivery of multiple smaller radiation doses over an extended treatment period.

The CA20948 rat pancreatic tumor is extremely aggressive, with a doubling time of 12–36 h. Stolz *et al.* (4) recently reported complete eradication of CA20948 tumors in 71% of rats treated with 10 mCi/kg (370 MBq/kg) of ^{90}Y-SMT 487 (^{90}Y-DOTA-Y3-OC), with no observable side effects. No absorbed dose measurements to the tumor or normal organs or specific blood chemistry/toxicity data were reported, however. The efficacy of ^{90}Y-SMT 487 has been reported in three patients (11), and this agent is presently in Phase I clinical trials in patients with somatostatin receptor-positive malignancies (3, 29). ^{90}Y has a mean β energy of 0.9 MeV with a maximum energy of 2.27 MeV and a maximum particle range of about 11 mm in tissue, making it an appropriate radionuclide for large tumor burdens. ^{64}Cu emits a 0.58-MeV β^- particle (40%), a 0.66-MeV β^+ particle (19%), and a γ photon of 1.34 MeV (0.5%), yielding a mean range of penetrating radiation of ~1.4 mm in tissue; therefore, ^{64}Cu emissions are more suitable for smaller tumor masses. In the ^{90}Y study, the tumor sizes were 12,805 ± 1140 mm^3 at the time of injection (4). In our studies, rats are sacrificed when the tumor reaches >10,000 mm^3 or the tumor ulcerates; thus, the tumors in our experiments were initially much smaller than those in the investigation of Stolz *et al.* (4). The size of the tumor at the beginning of the treatment may account for the difference in the response of the tumors to the different radionuclides; therefore, a meaningful comparison cannot be made between the efficacy of the ^{90}Y and ^{64}Cu compounds at this time. Moreover, the treatment of the tumor with ^{64}Cu-TETA-Y3-TATE may lead to the selective killing of receptor-rich cells. Theoretically, multiple dose schedules may cause a significant decrease in somatostatin receptor density after repeated administrations. As a consequence, regrowth of CA20948 tumors after treatment is possible because cells with a smaller number or no somatostatin receptors survive during ^{64}Cu-TETA-Y3-TATE treatment. Therefore, because of the longer mean range of the ^{90}Y β^- particle, receptor-negative bystanding cells could also be killed. This may also account, in part, for the complete eradication of CA20948 tumors reported by Stolz *et al.* (4).

In the 3 × 20 mCi dose study reported here, the treated rats gained weight throughout the experiment and at no time presented with any overt physical signs of toxicity, such as lethargy, scruffy coat, >10% weight loss, or diarrhea. Transient elevation and decrease in certain hematological and enzyme levels were noted, but by day 34 after the first treatment, all levels returned to baseline values. Although not fully comprehensive, these toxicity data are encouraging in that a maximum tolerated dose was not achieved and that larger quantities of radioactivity could be administered safely.

The biodistribution of ^{64}Cu-TETA-Y3-TATE in rats was clearly affected by the mass of peptide injected. A bell-shaped relationship has been reported between mass of ^{111}In-pentetreotide and its uptake in receptor-rich tissues (30). In this investigation, there was maximum uptake in somatostatin receptor-positive tissues at the lowest mass dose (10 ng), with de-

creasing uptakes in these tissues at higher masses. For the specific activities given in this report (1.25–2.5 mCi/μg), this would convert to 4–16 μg of material being injected in the 10- and 20-mCi treatment doses. This would result in the lowering of uptake of ^{64}Cu-TETA-Y3-TATE into receptor-rich tissues, which would have direct consequence on the regression of the tumor and dosimetry estimations. Using ^{64}Cu-TETA-Y3-TATE labeled at high specific activities may improve is therapeutic efficacy.

On the basis of the estimated absorbed doses from the baboon PET images, a typical injectate of ^{64}Cu-TETA-Y3-TATE for an imaging study will result in a total dose to the kidneys of 1.25 rad/mCi (0.337 mGy/MBq). This appears to be the dose-limiting organ, because the bladder dose measured in a baboon would be reduced >7-fold by a normal voiding scheme. The kidney dose determined from the nonhuman primate is ~3-fold higher than what was determined from rats. Dosimetry data presented for the intestinal tract also showed distinctly different results from the rat and baboon experiments. The different biodistribution between nonhuman primates and rodents is not surprising, given that hepatobiliary and renal clearance of many radiopharmaceuticals vary widely from rodents to mammals (31, 32). The decreased intestinal uptake and increase of renal dose in the baboon is likely to be more representative of human biodistribution.

Although the large discrepancies between rodent and primate biodistributions of radiopharmaceuticals are not surprising, the fact that human absorbed dose estimates of ^{64}Cu-TETA-Y3-TATE based on rat biodistribution data are greatly underestimated compared with doses obtained from baboon PET imaging data are problematic. Previous studies in our group on the dosimetry of ^{64}Cu-labeled monoclonal antibody 1A3-F(ab')$_2$ showed that absorbed dose estimates from rat biodistribution data overestimated what was found from baboon PET imaging data (33). Preliminary studies of ^{64}Cu-TETA-OC in patients showed that absorbed dose estimates based on rat biodistribution data were not greatly different from actual absorbed doses determined from human PET images.[4] Because of the distinct differences in the biodistribution of ^{64}Cu-TETA-Y3-TATE in rodents and primates, we will base future dosimetry estimates on radiolabeled somatostatin analogues from primate data prior to human studies.

Although human absorbed doses were not estimated in the radiotherapy studies reported previously using ^{188}Re and ^{90}Y-labeled somatostatin analogues in tumor-bearing mice (28, 34), dosimetry results have been reported in the two of the clinical case studies of ^{111}In-DTPA-octreotide therapy. In the report by Krenning *et al.* (8), the patient received a total of 550 mCi (20.4 GBq) over seven administrations, and the estimated doses to the liver and kidneys were 240 and 500 rad (2.4 and 5.0 Gy), respectively, whereas the estimated dose to the tumor was 1300 rad (13 Gy). Fjälling *et al.* (9) reported doses of 630 rads (6.3 Gy) to the liver (which had liver metastases), 371 rad (3.71 Gy) to the spleen, and 212 rad (2.12 Gy) to the kidney and red marrow. A study by Cremonesi *et al.* (35) used ^{111}In-DOTA-Y3-OC to estimate the absorbed doses that would be received in a therapy study with ^{90}Y-DOTA-Y3-OC. They reported the average estimated dose that would be given for a ^{90}Y therapy trial where 30 mCi (1.1 GBq) was administered per cycle for three cycles. The estimated absorbed doses due to ^{90}Y-DOTA-Y3-OC were 231 rads (2.31 Gy) to the liver, 2508 rad (25.08 Gy) to the spleen, 1089 rad (10.89 Gy) to the kidney, 9 rad (0.09 Gy) to red marrow, and 3333 rad (33.33 Gy) to the tumor. In the present study reported here, a single dose of 15.5 mCi of ^{64}Cu-TETA-Y3-TATE was administered to tumor-bearing rats, which weighed ~250 g. Extrapolating this to humans would suggest a total dose of about 4000 mCi (148 GBq) of ^{64}Cu-TETA-Y3-TATE for clinical therapy trials. On the basis of the nonhuman primate data, a delivered dose of 4000 mCi (148 GBq) of ^{64}Cu-TETA-Y3-TATE would result in absorbed doses of 5000 rad (50 Gy) to the kidney, 1560 rad (15.60 Gy) to the liver, 1400 rad (14 Gy) to the spleen, and 152 rad (1.52 Gy) to the red marrow. The kidneys are the critical organ in this study, and a reduction in kidney absorbed dose would be necessary. Methods have been used to decrease the uptake of radiolabeled proteins and peptides in the kidneys, in particular after the i.v. administration of D-lysine (13).

The absorbed dose to the CA20948 tumor from ^{64}Cu-TETA-Y3-TATE, calculated from the rat biodistribution, was 40.8 rad/mCi (11.0 mGy/MBq), compared with 30.9 rad/mCi (8.4 mGy/MBq) from ^{64}Cu-TETA-OC (7). It is important to note that this is the dose to the rat tumor and not an estimated dose to human tumors. By simple calculation, this represents a dose of 6120 mGy (612 rad) for ^{64}Cu-TETA-Y3-TATE to the CA20948 tumor in the single-dose experiment (1 × 15.5 mCi).

The data presented here clearly demonstrate that, for targeted radiotherapy with ^{64}Cu-TETA-Y3-TATE, the use of a multiple dose schedule is superior to single injections in terms of efficacy and toxicity. However, the effect of multiple doses on the tumor uptake of ^{64}Cu-TETA-Y3-TATE must be considered for each consecutive injection. Preliminary studies suggest that uptake of ^{64}Cu-TETA-Y3-TATE in receptor-positive organs and the CA20948 tumor decreases for subsequent identical injections given at 48-h intervals (36). In this study, it was shown that tissue uptake was less affected with the longer intervals between administrations (72 h) and suggests that longer dose fractionation protocols may be superior in therapeutic efficacy than 48-h treatment regimens. Future studies in tumor-bearing rats will include the use of MicroPET imaging (Concorde Microsystems, Knoxville, TN) to determine the optimal time interval for multiple dose regimens. PET imaging will enable the calculation of biodistribution data (*i.e.*, dosimetry measurements) and the determination of therapeutic efficacy simultaneously.

In conclusion, ^{64}Cu-TETA-Y3-TATE was effective in causing tumor regression of CA20948 tumors in rats. A multiple dose regimen of ^{64}Cu-TETA-Y3-TATE temporarily eradicated CA20948 tumors, without lethal toxicity to the animal. It is clear that optimization of the radiotherapeutic multiple dose regimen is necessary to improve upon the results presented in this report. The results reported here also showed significant discrepancies between absorbed dose estimates obtained from rat and baboon biodistribution. These data suggest that ^{64}Cu-TETA-Y3-TATE may not be optimal agent for targeted radiotherapy but does,

[4] C. J. Anderson *et al.*, manuscript in preparation.

however, confirm that other ^{64}Cu-labeled somatostatin analogues warrant continued consideration as agents for targeted radiotherapy.

ACKNOWLEDGMENTS

We thank Dr. Deborah W. McCarthy and Todd A. Perkins for production of ^{64}Cu and Elizabeth L. C. Sherman, Lynne A. Jones, Lennis Lich, and Jason Brandstetter for excellent technical assistance.

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Nuclear Medicine and Biology 28 (2001) 327–334

NUCLEAR MEDICINE — AND — BIOLOGY

Radiotherapeutic efficacy of ^{153}Sm-CMDTPA-Tyr^3-octreotate in tumor-bearing rats☆

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Received 13 August 2000; received in revised form 4 November 2000; accepted 30 December 2000

Abstract

A number of radiolabeled somatostatin analogs have been evaluated in animal tumor models for radiotherapeutic efficacy. The majority of the agents tested have used either high-energy beta-emitters, such as Y-90 or Re-188, or the Auger electron-emitting radionuclide, In-111. Because a medium-energy beta-emitter might have equivalent efficacy compared to high-energy emitters, and lower toxicity to non-target tissues, we have evaluated the therapeutic potential of the beta-emitting nuclide, Sm-153, chelated to the somatostatin analog, CMDTPA-Tyr^3-octreotate. Using an in vitro binding assay, this octreotate derivative was shown to have high affinity for the somatostatin subtype-2 receptor (IC_{50} = 2.7 nM). Biodistribution studies in CA20948 tumor-bearing Lewis rats demonstrate that the Sm-153 labeled compound has high uptake and retention in tumor tissue (1.7% injected dose/g tissue, 4 hrs post injection) and has rapid overall clearance properties from non-target tissue. Radiotherapy studies were carried out using ^{153}Sm-CMDTPA-Tyr^3-octreotate and CA20948 tumor bearing Lewis rats at 7 days post implant. Dose regimens consisting of single and multiple i.v. injections of 5.0 mCi/rat (185 MBq) were employed over a time span of 7 days. Suppression of tumor growth rate was observed in all treated animals compared to untreated controls. Greater inhibition of tumor growth was observed in animals that received multiple doses. These studies indicate that medium-energy beta-emitting isotopes have considerable potential for the treatment of somatostatin receptor-positive tumors.

Keywords: Octreotate; Octreotide; Samarium; Somatostatin; Peptides; Radiotherapy

1. Introduction

The use of peptides as a means of targeting radionuclides to specific tissues has recently become more promising in both diagnostic and therapeutic applications. The wider use and acceptance of small peptide-based molecules has resulted from the ability to design compounds that have favorable biological characteristics, such as rapid blood clearance, low toxicity, high uptake into tumor tissue, and low immunogenicity [3,30,37,38]. The clinical use of OctreoScan® (^{111}In-DTPA-octreotide) is a specific example of a peptide-based imaging agent. The development of this diagnostic agent for use as a targeted radiotherapeutic has been reported [8,16,17,19,33]. Various octreotide derivatives radiolabeled with isotopes, including Y-90, and Re-188, have been reported to inhibit tumor growth in animals [16,33,38,39]. While high-energy (>1–2 MeV) beta-emitters have been used in these studies, there may be advantages to using lower energy beta-emitting radionuclides (<1 MeV). A low-energy particle should still be sufficient to kill targeted (and nearby) tumor cells but would be less damaging to non-target tissues. In this study, the radionuclide samarium-153 was used to test this premise.

The potential application of Sm-153 in peptide-targeted radiotherapy is indicated by its successful use in both bone palliation [4,13,22,24] and in radiation synovectomy [11, 20,22,23]. Sm-153 ($T_{1/2}$ = 46.3 hr) is a medium-energy beta-emitter (0.808 MeV, 17.5%; 0.705 MeV, 49.6%; 0.635 MeV, 32.2%) with a 30% abundance gamma emission (103 keV) that is suitable for use in scintigraphy [23]. In addition

☆An abstract containing a portion of the work presented in this paper was presented at the SNM meeting in Los Angeles, June 1999: J.E. Bugaj, J.L. Erion, M.A. Schmidt, R.R. Wilhelm, S.I. Achilefu, A.J. Srinivasan, J. Nucl. Med. (40) (1999) 223.

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Abbreviations: ACN, acetonitrile; TFA, trifluoroacetic acid; CMDTPA, carboxymethyldiethylenetriaminepentaacetic acid; sst_2, somatostatin subtype-2 receptor

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PII: S0969-8015(01)00197-4

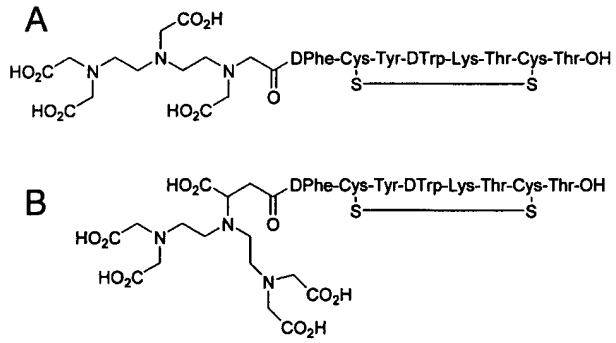

Fig. 1. Structures of (A) DTPA-Tyr^3-octreotate and (B) CMDTPA-Tyr^3-octreotate.

to the desirable emission properties of Sm-153, this lanthanide also readily forms complexes with DTPA-type chelates that can be conjugated to small peptide based molecules [32].

In previous work [21], it was demonstrated that even small variations in the structure of a DTPA-type ligand could have significant effects on the biodistribution properties of a Sm-153 labeled somatostatin peptide derivative. In other studies, we have shown that a substitution of the carboxy-terminal threoninol in octreotide with the natural amino acid threonine to generate a derivative termed, octreotate, substantially enhances the uptake and retention of a radiolabeled derivative in somatostatin receptor-expressing tumors [17,21]. In this study, we evaluated the therapeutic potential of one of these optimized derivatives, ^{153}Sm-CMDTPA-Tyr^3-octreotate (Fig. 1), in a rat tumor model.

2. Materials and methods

2.1. Preparation of somatostatin analogs

Solid phase peptide synthesis of CMDTPA-Tyr^3-octreotate and other octreotate derivatives was carried out with an Applied Biosystems 432A *Synergy* peptide synthesizer (Foster City, CA) employing Fmoc (9-fluorenylmethoxycarbonyl) strategy, utilizing 25 μmol of Fmoc-Thr(OtBu) Wang resin and 75 μmol of subsequent Fmoc-protected amino acids. Activation was accomplished using N-hydroxy-benzotriazole (HOBt) and 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). Penta-*t*-butyl carboxymethyl DTPA (protected CMDTPA) or tri-*t*-butyl DTPA was incorporated at the N-terminus in the same manner. Penta-*t*-butyl CMDTPA was prepared by a modification of a procedure described by Williams and Rappaport [35]. These authors carried out the bis *N*-alkylation of *p*-nitrophenylalanine benzyl ester with di-*t*-butyl [*N*-(bromoethyl)amino]diacetate using a phosphate buffer/acetonitrile reaction medium. This phenylalanine derivative was substituted here with L-aspartic acid β-benzyl, α-*t*-butyl ester. The benzyl ester of the resulting product was then cleaved via hydrogenolysis in methanol using 10% Pd/C as the catalyst to generate penta-*t*-butyl CMDTPA. Tri-*t*-butyl DTPA was prepared according to our previously published procedure [31]. Upon completion of the linear peptide, disulfide cyclization was performed manually by suspension of the resin-bound peptide in DMF (2 mL) containing 75 μmol thallium(III) trifluoroacetate (3 hr reaction at room temperature). After washing the resin with DMF and THF, the peptide was cleaved from the resin and deprotected with 85% trifluoroacetic acid/5% thioanisole/5% phenol/5% water (6 hrs). The peptide was precipitated with 10 mL t-butyl methyl ether and centrifuged. The peptide/resin pellet was then washed with 4 × 10 mL of t-butyl methyl ether. Acetonitrile/water (2:3) was finally added to dissolve the peptide, and the mixture was filtered to remove the resin. Crude peptide solutions were lyophilized prior to purification by HPLC. Final purification was accomplished by reverse phase HPLC on a Vydac C18 column using an acetonitrile/water gradient containing 0.1% TFA. Molecular weights were determined by mass spectrometry operating in the electrospray ionization mode.

2.2. Radiochemistry

$^{153}SmCl_3$ in 0.05N HCl was obtained from MURR (Columbia, MO) at a specific activity of ~700 Ci/mmol (25.9 TBq/mmol). Radiolabeling was carried out in 25 mM NaOAc, 12.5 mM sodium ascorbate (pH 5.0, room temperature). Small-scale reactions (10 mCi) were carried out by addition of 25 μL of $^{153}SmCl_3$ (400 mCi/ml in 0.05 N HCl) to 50 μL buffer (50 mM NaOAc, 25 mM sodium ascorbate) and 25 μL CMDTPA-Tyr^3-octreotate (1.1 molar excess peptide: Sm, 30 to 45 μg peptide in water). Reactions were incubated at room temperature for 30 min. For larger scale reactions (50 mCi), proportions identical to the small-scale reaction were used. The specific activity of radiolabeled peptide ranged from 300 to 500 Ci/mmol (11.1–18.5 TBq/mmol). For in vivo studies, ^{153}Sm-CMDTPA-Tyr^3-octreotate was diluted to 10 mCi/ml (370 MBq/ml) in phosphate-buffered saline containing 5% ethanol. Radiochemical yield (typically >98%) and purity (>95%) were determined by reverse phase HPLC on a Nova-Pak C18 column, 3.9 × 150 mm (Waters, MA), using a 15-min linear gradient of 0 to 70% solvent B at 1 ml/min (solvent A, 5% ACN/25 mM triethylamine pH 6.0; solvent B, 90% ACN/25 mM triethylamine, pH 6.0). The ^{153}Sm radiolabeled peptide eluted with a retention time of 13.2 min.

2.3. Receptor binding assays

Receptor binding assays were performed using membranes prepared from CA20948 tumors harvested from implanted Lewis rats [3]. Assays were carried out using the Millipore Multiscreen system (Bedford, MA) with ^{111}In-DTPA-Tyr^3-octreotate as the trace and unlabeled CMDTPA-Tyr^3-octreotate or other peptide derivatives as

the cold competitor. Radiolabeling of DTPA-Tyr^3-octreotate was performed by combining 1.0 μg peptide (1.0 mg/ml) with 1.0 mCi of $^{111}InCl_3$ (100 mCi/ml, Mallinckrodt Inc., St. Louis) and 10 μL buffer (50 mM NaOAc, 25 mM sodium ascorbate). After incubation at room temperature for 15 minutes, the peptide radiochemical yield (>99%) and purity (>95%) were determined by reverse phase HPLC. IC_{50} values were calculated using a four-parameter curve fitting routine using the program GraFit (Erithacus Software, UK).

2.4. Animal tumor model

All animal studies were conducted in compliance with the Mallinckrodt Inc. Animal Welfare Committee requirements. Male Lewis rats (120–140 g) were purchased from Harlan (Indianapolis, IN). The somatostatin subtype-2 (sst_2) receptor positive pancreatic acinar tumor line, CA20948, was maintained by serial passage in Lewis rats [3,27,28]. Tumor tissue was implanted into the left flank of the animal, and after approximately 2 weeks, tumor volumes were adequate for use in biodistribution studies. For therapy studies, tumor-implanted animal were used at 7 days post implant, at which time, tumors were palpable.

2.5. Tissue biodistribution studies

Biodistribution studies were carried out using male Lewis rats bearing CA20948 tumors (20-days post implant). Anesthetized (Metofane) animals received 25 μl (25 μCi [0.925 MBq]) of radiolabeled compound via the jugular vein. The animals were sacrificed in three groups (n = 3) at 1, 4, and 24 hours post injection by cervical dislocation. The tissues and organs of interest were removed, weighed, and radioactivity measured in a Packard Cobra gamma-scintillation counter.

2.6. Radiotherapy studies

CA20948 tumor tissue was implanted into the left flank of Lewis rats as described above. At 7-days post implant, animals were randomly divided into three study groups. The presence of a tumor was confirmed in all animals by palpation of the animal's left flank. For each treatment group a set of negative control tumor-implanted animals (n = 6/group) was maintained. Negative control animals received no treatment, and the three treated groups receiving ^{153}Sm-CMDTPA-Tyr^3-octreotate were as follows: (1) single i.v. dose of 5.0 mCi (185 MBq), (2) three doses of 5.0 mCi each with 7-day intervals between doses, (3) five doses of 5.0 mCi each with 7-day intervals between doses. Tumor volume measurements were performed on a weekly basis and calculated using the formula for an ellipsoid ($v = \pi/6[l \cdot w \cdot h]$).

Dosimetry calculations are based on the residence times of the radiolabeled peptide in organs and tumor tissue determined from biodistribution data, and the percent dose uptake per gram tissue. MIRD s-values were calculated assuming spherical geometry using energy tables from the Lawrence Berkeley National Laboratory database [10]. Tumor and organ doses correspond to self-absorbed dose only and do not include contributions from other organs.

Gamma scintigraphy was performed using a Picker 300 SX gamma camera interfaced to a dedicated Odyssey Imaging Processor. The gamma images of the rats injected with Sm-153 were obtained with a large field of view camera fitted with a general-purpose low-energy collimator for 100 K counts with the peak energy centered at 103 keV. The same conditions were used for obtaining In-111 images with the exception that the collimator was a medium-energy collimator, and the peak energies were centered at 171 and 245 keV. When used in this latter configuration, the gamma-camera does not detect Sm-153 gamma emissions. All rats were imaged in the prone position.

3. Results

3.1. In vitro assays

The CA20948 prancreatic acinar tumor line expresses high levels of the sst_2 receptor [3,17,28,33]. We have used membrane preparations from this tumor line in an in vitro assay to screen a number of somatostatin analogs with chemical modifications made in both the peptide and chelate segment of the molecule. Fig. 2 shows the data for several compounds analyzed using this assay. The estimated IC_{50} for CMDTPA-Tyr^3-octreotate was found to be essentially the same as that determined for DTPA-octreotide (2.8 and 2.5 nM, respectively). This compound was therefore selected as a promising candidate for further testing because of its high binding affinity and improved samarium chelating properties [21].

3.2. Tissue biodistribution study

The tissue biodistribution properties of ^{153}Sm-CMDTPA-Tyr^3-octreotate were studied in CA20948 tumor-bearing Lewis rats (Table 1, Fig. 3). At 1, 4, and 24 h post injection (p.i.) of the compound the amount of radioactivity that accumulated in the tumor tissue was 1.45, 1.73, and 0.74% of the injected dose per gram (%ID/g). Significant uptake was also observed in the pancreas and adrenal glands, both sst_2 receptor positive tissues. Pancreas uptake was 3.43, 3.02, and 1.74 %ID/g, and the adrenal tissue was 0.18, 0.17, and 0.15% of the injected dose per whole organ (%ID/o) at 1, 4, and 24 h p.i., respectively. Non-somatostatin receptor expressing tissues including the liver, muscle, spleen, and heart exhibited low accumulation of the radiotracer. The kidneys were the only non-receptor positive tissue in which high uptake was observed (3.34, 3.03 and 2.51% ID/gram; 1, 4, 24 h respectively). Scintigraphs at all

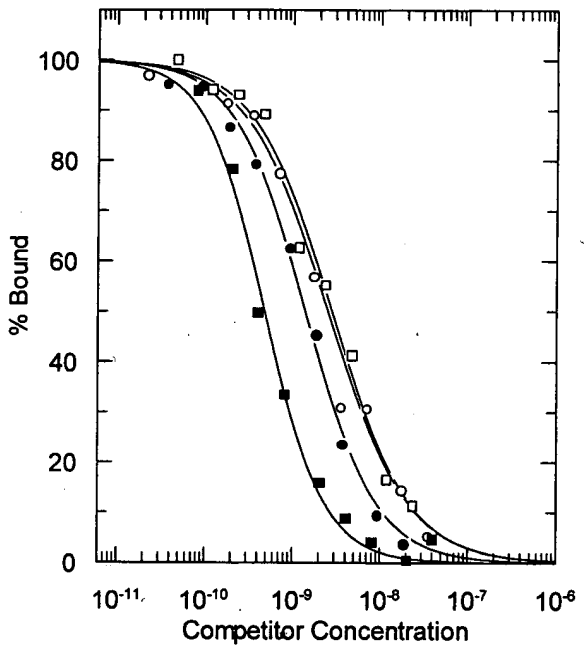

Fig. 2. Graph of competition binding of ^{111}In-DTPA-Tyr^3-octreotate to CA20948 tumor membranes in the presence of increasing concentration of unlabeled somatostatin analogs: ●, DTPA-Tyr^3-octreotate; ○, DTPA-octreotide; □, CMDTPA-Tyr^3-octreotate; ■, Tyr^3-octreotate. The corresponding calculated IC_{50} values are 1.39 nM, 2.51 nM, 2.77 nM, and 0.48 nM, respectively.

time points confirmed the strong localization of the agent in the tumor with no significant uptake observed in other non-sampled tissues or organs (data not shown). At 24 h, the predominant route of clearance was urinary (75.2%) with only 4.5% of the radioactivity recovered in the feces. The rapid distribution and excretion of this agent resulted in high target to non-target tissue ratios, except as noted for the kidneys. The tumor to tissue ratios calculated from %ID/g values at 24 h p.i. were 737 for blood, 369 for muscle, 9 for liver, and 0.3 for kidneys.

3.3. Radiotherapy

Preliminary studies were carried out to assess the dose levels required for ^{153}Sm-CMDTPA-Tyr^3-octreotate to affect the growth rate of CA20948 tumors in Lewis rats. It was found that relatively small doses (0.5 mCi/animal) measurably reduced the growth rate of tumors, but that higher doses (3.0–5.0 mCi) were necessary to either decrease tumor volumes or significantly delay tumor regrowth (data not shown). To further determine the therapeutic potential of this agent, three separate dosing regimens were tested using a 5.0 mCi dose with one, three, and five treatments. Treatments were spaced 7 days apart because the biological clearance of the agent was essentially complete by this time. The effect of repeat doses on the biodistribution of the radiolabeled peptide was not determined. However, an evaluation of the mass effects of peptide (6 ng to 250 μg) on the biodistribution of ^{111}In-DTPA-Tyr^3-octreotate (data not shown) indicates that the amount of peptide used in each treatment (~15 μg) was insufficient to block the uptake of a subsequent dose by more than 50% of levels found in a normal biodistribution study [17]. Similar results have been demonstrated by others [7,18] with DTPA-octreotide. Our data also show that when tumors re-emerged they continued to express high levels of the somatostatin receptor (Fig. 5B and C). Additionally, as determined from the aforementioned mass effects study, it was not feasible to compare the effect of a single cumulative dose with the multiple dose regimens because the level of peptide required for 15 and 25 mCi doses at 500 Ci/mmol (40 to 70 μg) would significantly attenuate the uptake of radiolabel into tumor tissue. An untreated control group of tumor-implanted animals (n = 6) was maintained for each treatment group to limit uncertainties arising from possible variability in tumor growth rates or in somatostatin receptor expression following serial passages of tumor tissue.

Fig. 4 shows the results of the single and multiple dose administration of ^{153}Sm-CMDTPA-Tyr^3-octreotate in tumor-bearing Lewis rats. In all three studies, untreated tu-

Table 1
Biodistribution of ^{153}Sm-CMDTPA-Tyr^3-octreotate in CA20948 tumor-bearing Lewis rats (%ID/gram tissue, n = 3)

	Time post injection		
Tissue sample	1 Hour	4 Hours	24 Hours
Blood	0.083 ± 0.011	0.006 ± 0.001	0.001 ± 000.0
Liver	0.072 ± 0.008	0.079 ± 0.011	0.084 ± 0.007
Kidneys	3.344 ± 0.454	3.031 ± 0.112	2.510 ± 0.190
Skeletal Muscle	0.018 ± 0.002	0.004 ± 000.0	0.002 ± 0.001
Spleen	0.042 ± 0.004	0.036 ± 0.013	0.030 ± 0.004
Heart	0.044 ± 0.004	0.011 ± 0.001	0.007 ± 000.0
Pancreas	3.425 ± 0.264	3.023 ± 0.082	1.736 ± 0.110
Small Int	0.307 ± 0.077	0.395 ± 0.132	0.125 ± 0.011
Stomach	0.275 ± 0.047	0.392 ± 0.044	0.107 ± 0.011
Bone	0.138 ± 0.012	0.129 ± 0.010	0.134 ± 0.005
Tumor	1.453 ± 0.371	1.726 ± 0.332	0.737 ± 0.053

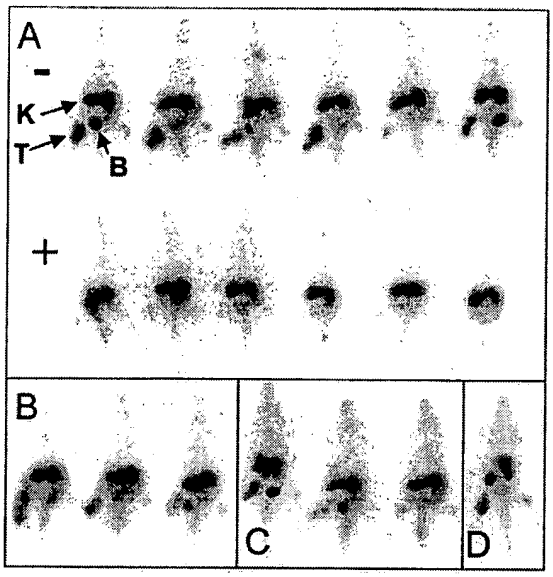

Fig. 5. Gamma-scintigraphy of treated or untreated CA20948 tumor-implanted Lewis rats. A, scintigraphs of rats treated with 3 × 5 mCi (+) of ^{153}Sm-CMDTPA-Tyr^3-octreotate and the corresponding control group (−) were imaged at 30 days post tumor implant. Symbols on scintigraphs indicate position of (T) tumor, (K) kidney, and (B) bladder. B, scintigraphs of 3 of 5 surviving rats treated with a single 5 mCi dose of ^{153}Sm-CMDTPA-Tyr^{3}octreotate at 37 days post treatment. C, scintigraphs of 3 of 6 surviving rats treated with 5 × 5 mCi doses of ^{153}Sm-CMDTPA-Tyr^3-octreotate at 78 days post tumor implant. A, B, C, Animals were injected with 50 μCi of ^{111}In-DTPA-Tyr^3-octreotate and imaged at 3 hours post injection. D, scintigraph of tumor bearing Lewis rat (3 wks post implant) at 3 hrs post injection of 250 μCi ^{153}Sm-CMDTPA-Tyr^3-octreotate.

were consistent with the observed tumor uptake of ^{111}In-DTPA-Tyr^3-octreotate suggesting uptake was not compromised by receptor saturation from previous doses. Multiple treatments improved efficacy over a single 5 mCi dose; at 95 days post tumor implant there were 50% surviving rats treated with 3 × 5 mCi and 100% surviving rats treated with 5 × 5 mCi. Tumor regrowth was, however, observed in all treated animals with no animal surviving more than 125 days post tumor implant (Fig. 4). In all cases, the tumors that eventually developed in animals treated with either single or multiple doses still expressed high levels of the sst_2 receptor as shown in scintigraphs of animals imaged with ^{111}In-DTPA-Tyr^{3}octreotate (Fig. 5B and C). Dosimetry estimates for the tumor and organs with significant uptake were calculated using the percent uptake and residence times determined from the biodistribution data. For a single 5 mCi treatment, doses to the tumor, kidney and pancreas are calculated to be 8.7 Gy, 29.5 Gy, and 18.7 Gy respectively.

4. Discussion

The studies presented here demonstrate the radiotherapeutic potential of the medium-energy beta-emitting radionuclide, Sm-153, chelated to the somatostatin analog CMDTPA-Tyr^3-octreotate. This agent's high affinity for the somatostatin sst_2 receptor and its long-term retention in tumor tissue are properties that are required for effective radiotherapy. The biodistribution data of ^{153}Sm-CMDTPA-Tyr^3-octreotate in CA20948 tumor-bearing Lewis rats showed that this compound has high uptake and retention in tumor tissue expressing the sst_2 receptor. The tumor uptake is greater than that observed for ^{111}In-DTPA-octreotide [16, 17], and is similar to that reported for ^{90}Y-DOTA-Tyr^3-octreotide [16]. Higher tumor uptake has been reported for ^{111}In-DTPA-Tyr^3-octreotate [17], but we found the corresponding ^{153}Sm-DTPA complex to be somewhat less stable in vivo than the ^{153}Sm-CMDTPA complex. The tumor uptake of the DTPA- or CMDTA-Tyr^3-octreotate compounds radiolabeled with Sm-153 was not significantly different (data not shown). There was low uptake/retention of ^{153}Sm-CMDTPA-Tyr^3-octreotate in most non-target tissues with only the observed kidney uptake representing a limiting factor to dose escalation [1]. It has been demonstrated, however, that a significant reduction of the kidney retention of other similar somatostatin metal-chelate analogues can be achieved by the use of amino acid i.v. infusion [5,17]. The pancreas is an sst_2 receptor positive organ in which potentially undesirable uptake was observed. Additional studies are necessary to determine whether the high pancreatic uptake observed in rats will also be found in humans. Results with other octreotate derivatives in primates, where no apparent pancreas uptake is observed in scintigraphs, suggest that this will not be the case [25]. Moreover, all animals from the high multiple dose treatment group were examined at death and no visual signs of tissue pathology were noted in kidneys or pancreas. Other overt parameters such as weight gain and grooming behavior during the course of the study were also normal in these animals. In addition to the overall favorable biodistribution properties of ^{153}Sm-CMDTPA-Tyr^3-octreotate, the compound also has excellent clearance properties with almost 80% of the injected dose excreted within 24 hours, predominately via the renal system.

The radiotherapeutic efficacy of ^{153}Sm-CMDTPA-Tyr^3-octreotate is demonstrated here by our finding that even a single 5 mCi dose per animal was sufficient to attenuate tumor growth as compared to untreated controls. More pronounced suppression of tumor regrowth was observed when multiple dose regimens were administered. With three or five doses of 5 mCi at one-week intervals, we observed a mean survival of 101 days post tumor implant as compared to <40 days for untreated controls. In all cases, however, treated rats ultimately succumbed to regrowth of latent tumor cells. Despite this, the therapeutic effect was significant and similar or superior to results achieved by workers using other radiolabeled somatostatin analogs. For example, Anderson *et al.* [3] reported the therapeutic effect of ^{64}Cu-TETA-octreotide in the same tumor model employed here. In their studies, treatment of tumor-bearing rats

with one or two, 15 mCi doses of radiolabeled peptide also resulted in delayed tumor growth; however, at two weeks post treatment, tumor volumes in treated animals were comparable to untreated controls [3]. De Jong *et al.* reported a transient tumor response in rats (CA20948 tumors) following treatment with 2 × 3 mCi of ^{90}Y-DOTA-Tyr^3-octreotide with rats surviving 65 days on average post treatment [19]. Using the latter agent in the same animal tumor model, Stolz *et al.* reported complete remission in 5 of 7 animals with a single treatment of 2.5 mCi per animal [33]. These data indicate that identical tumor models can behave differently when maintained in separate laboratories. The variables that alter tumor radiosensitivity, such as volume and growth rate, which may account for the discrepancies, have not yet been evaluated.

5. Conclusion

The recent search for the ideal radionuclide for targeted radiotherapy using somatostatin analogs has resulted in the evaluation of Y-90, Cu-64, In-111, Re-188, I-131, and now Sm-153 [2,3,17,19,33,38,39]. The beta emission energy of Sm-153 is similar to that of I-131, an extensively used therapeutic radionuclide. Sm-153 also emits a partial low-energy gamma emission (30%, 103 keV), which allows for scintigraphic imaging, useful in staging radionuclide therapy and performing dosimetric calculations. A low or medium-energy beta emission as compared to the high-energy emissions of Y-90 or Re-188, for example, is expected to be less toxic to critical non-target organs. Additionally, peptide degradation due to radiolysis is easier to prevent in compounds radiolabeled with Sm-153 than with high-energy beta-emitters. Though there are advantages to using Sm-153, the relatively low specific activity (~700 Ci/mmol) results in some limitations, especially if an agonist-targeting agent is used, which may have negative physiological effects at high concentrations. Additional studies are necessary to determine whether these limitations can be overcome, or whether they can be circumvented using other low to medium-energy beta-emitting radionuclides.

Acknowledgments:

The authors thank Randy Wilhelm for mass spec analysis of peptides, and Dr. C.J. Anderson, Washington Univ., St. Louis, for seed CA20948 tumor tissue.

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CANCER BIOTHERAPY & RADIOPHARMACEUTICALS
Volume 14, Number 6, 1999
Mary Ann Liebert, Inc.

Receptor-mediated Radionuclide Therapy with ^{90}Y-DOTA-D-Phe^{1}-Tyr^{3}-Octreotide: Preliminary Report in Cancer Patients

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Recent advances in receptor mediated tumor imaging led to the development of a new somatostatin analogue DOTA-D-Phe^{1}-Tyr^{3}-Octreotide. This new compound, named DOTATOC, has shown high affinity for somatostatin receptors, stable labeling with yttrium-90 (^{90}Y) and favourable biodistribution in patients. The aim of this work was to evaluate acute and late toxicity and the response rate in cancer patients administered ^{90}Y-DOTATOC.

Twenty patients received three equal i.v. injections of ^{90}Y-DOTATOC. Cohorts of 5 patients were treated starting with 1.1 GBq per cycle in escalating dosage (0.4 GBq increments) in subsequent groups.

No patients showed acute or delayed major adverse reactions up to the dose of 2.2 GBq of ^{90}Y-DOTATOC per cycle (6.6 GBq total). Maximum tolerated dose has not been determined yet. One patient, after 4.4 GBq total dose, developed delayed kidney grade II toxicity. Complete and partial tumor mass reduction (CR and PR) was measured in 25% of patients along with 55% showing stable disease (SD) and 20% progressive disease (PD).

These results indicate that high activities of ^{90}Y-DOTATOC can be administered with low risk of myelotoxicity, although the radiation doses to the kidneys require careful consideration. Tumor doses were high enough in most cases to obtain objective therapeutic responses.

Key Words: Peptide-receptor radiotherapy, somatostatin analogue, ^{90}Y/^{111}In-DOTATOC.

INTRODUCTION

Different tumors, classically defined as neuroendocrine, contain high concentrations of type 2 somatostatin receptors (SSTR2) suitable to in-vivo localization of the tumors and their metastases using In-111 labeled somatostatin analogue scintigraphy.[1] Besides neuroendocrine tumors, somatostatin receptors have also been identified in tumors of the central nervous system,[2] breast,[3] lung and lymphatic tissue.[4] These observations served as the biomolecular basis for the clinical use of radiolabeled somatostatin analogues which, at present, are of great interest in oncology for diagnostic and peptide receptor radionuclide therapy applications.[5,6] A somatostatin analogue containing the metal chelator DOTA, [DOTA-D-Phe^{1}-Tyr^{3}]octreotide, named DOTATOC, has shown favourable characteristics for therapeutic

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use: high affinity for somatostatin subtype receptors SSTR2 and SSTR5,[7,8] high hydrophilicity[9] and ease of labeling and stability with In-111 and with Y-90.[10-13] In the present study, we report preliminary evaluations on toxicity and therapeutic efficacy of ^{90}Y-DOTATOC in patients.

MATERIALS AND METHODS

Patient Population

Twenty eligible patients (12 males and 8 females; age range 39-72 years) referred to us after conventional treatments for histologically confirmed malignant tumors (17 carcinoids, 1 breast cancer, 1 medullary thyroid cancer, 1 grade III meningioma), were enrolled in a protocol for receptor mediated radionuclide therapy (RMRT) with ^{90}Y-DOTATOC. All patients had measurable disease on computed tomography (CT), magnetic resonance imaging (MRI) or ultrasound. Inclusion criteria were: a) a positive diagnostic scan with ^{111}In-DOTATOC with favourable biodistribution and dosimetry; b) white blood cells > 2,500/m^3, haemoglobin > 10 g/dl, platelets > 1000,000/m^3, bilirubin < 2.5 mg/dl; (f) creatinine <1.5.

Exclusion criteria were: (a) pregnancy or lactation; (b) age < 21 years; (c) Karnofsky performance status < 60; (d) life expectancy <6 months; (e) presence of a known second neoplasm.

The study was performed after approval by the European Institute of Oncology Ethical Committee and all patients were informed of the nature, aim and potential risks of the study and signed a consent form before starting therapy.

Reagents

The somatostatin analogue [DOTA-D-Phe^1-Tyr^3] octreotide (DOTATOC; DOTA: 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid) was synthesized at the Institute of Radiological Chemistry of Basel University.[10,14] ^{90}Y chloride was purchased from AEA Technology (Harwell, UK). Typically, to 30 μg of DOTATOC in 30 μl 0.2M ammonium acetate (pH 5.0) were added 150 μl of 0.4M ammonium acetate/gentisic acid (pH 5.0) and 1.1 GBq $^{90}YCl_3$ (22.2 GBq/ml, 0.04M HCl) and the mixture was heated for 25 min at 90 °C. Quality assurance of ^{90}Y-DOTATOC was performed with the use of a Sep-Pak C18 cartridge (Waters, Millipore, MA, USA) and high-performance liquid chromatography (HPLC) as previously described.[15] Labeling yields more than 98% were routinely achieved at specific activity more than 50 GBq/μmol.

Administration Protocol

The ^{90}Y-DOTATOC in 100 ml of physiological saline was injected intravenously (i.v.) over a period of 10-15 minutes.

A horizontal protocol, consisting of a sequence of three equal administrations in the same patient, was followed. Briefly, the first 5 patients received 30 μg of DOTATOC labeled with 1.1 GBq of ^{90}Y for three cycles in a period of 6 months. A second group of 5 patients received 40 μg of DOTATOC labeled with 1.5 GBq of ^{90}Y for the same number of cycles. The other two groups of 5 patients were treated with 50 μg of DOTATOC labeled with 1.8 GBq of ^{90}Y and 60 μg of DOTATOC labeled with 2.2 GBq of ^{90}Y respectively.

The patients were hospitalized for 2-3 days after the therapeutic dose. Laboratory tests were performed prior to therapy and thereafter every two weeks to evaluate haematological changes, liver and renal function.

Biodistribution and Dosimetry

In those patients for whom tumor mass could be accurately evaluated by CT or MRI, whole body imaging was performed at 30', 3-4 h, 24 h and 48 h after injection of ^{111}In-DOTATOC, using a double-head gamma-camera (GE MAXXUS) equipped with a medium-energy general purpose collimator. SPECT studies of the tumors were also obtained 3-4 h after injection and visually matched with CT and MRI. Regions of interest were manually drawn over the total body, tumor and normal organs: heart, lungs, liver, spleen and kidneys. Data from the gamma-camera were converted to biological time-activity curves (%IA_{biol} (t)) taking into account background, attenuation and physical decay. To evaluate the kinetics of the system, a compartmental model was developed[16] and the SAAM II program[17] was used to fit the observed data in the compartmental model. When an acceptable fit was obtained,

the program was also used to determine the residence times for ^{111}In and ^{90}Y in the various source organs, with the assumption that the kinetics of DOTATOC with either ^{111}In or ^{90}Y would be identical.[10,18] Dose calculations were performed according to the MIRD formalism, entering the residence times for all source organs in the MIRDOSE 3.1 software and selecting either the standard man or woman phantoms, as appropriate to the gender and weight of the individual patient in order to reduce the approximation of considering standard organs.[19]

Toxicity and Therapeutic Effect

Toxicity and response rate was evaluated according to the WHO criteria.[20] The objective therapeutic response was evaluated on the basis of instrumental data such as CT and/or MRI, ecotomography to document the volume and the characteristics of the lesions before and after treatment. The post-therapy evaluation was performed 30-40 days after the final treatment.

RESULTS

Biodistribution and Dosimetry

A typical sequence of whole-body anterior scintigraphic images from 30 min up to 48 h after injection of ^{111}In-DOTATOC is shown in Fig.1. The rapid clearance of the tracer from the bloodstream is evident from the images confirming previous data on the pharmacokinetics of ^{111}In-DOTATOC where the cumulative activity excreted in the urine was 52%±12%IA (injected activity) at 4h and 73%±11%IA at 24h post-injection.[21]

The high tumor uptake allowed tumor detection as early as 3 h post-injection and the localization of the tracer remained very intense in the following images.

The observed biokinetics in the source organs with ^{111}In-DOTATOC were used to calculate residence times for ^{90}Y-DOTATOC.

The highest absorbed doses were to the spleen (7.6 ± 6.3 mGy/MBq) and to the kidneys (3.3 ± 2.2 mGy/MBq). The estimated absorbed dose to the red marrow was 0.03 ± 0.01 mGy/MBq. The absorbed dose to other tissues of the body was approximately 0.08 ± 0.04 mGy/MBq; total body dose was 0.14 ± 0.06 mGy/MBq.

The mean value of t_{tumor} for ^{111}In-DOTATOC was 0.5 h, ranging from 0.03 to 6.5 h and the predicted absorbed dose for ^{90}Y-DOTATOC was estimate to be 10.1 mGy/MBq, ranging from 1.4 to 31.0 mGy/MBq. Based on these calculations tumors were supposed to receive a mean dose between 33-66 Gy for a cumulative injected dose ranging from 3.3 to 6.6 GBq.

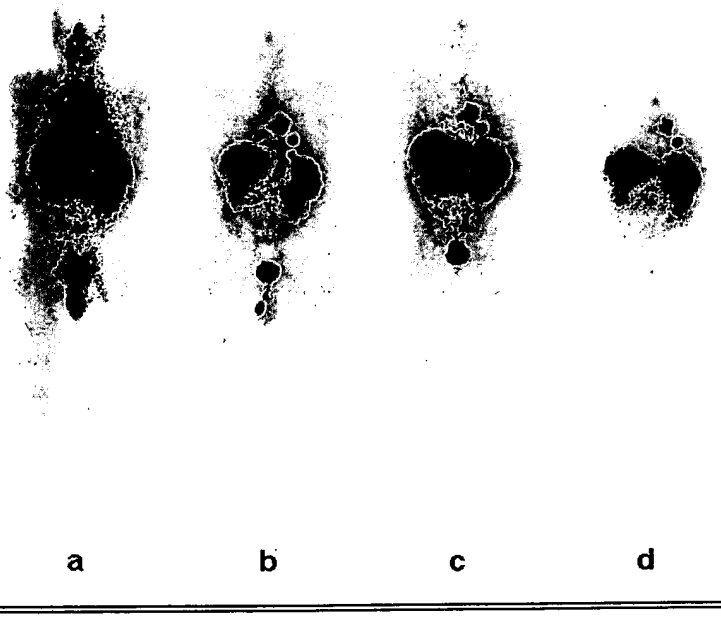

FIGURE 1: Whole-body anterior scintigraphic images after injection of ^{111}In-DOTATOC in a patient with left bronchous carcinoid and liver metastases. Note the rapid clearance of the tracer and the excellent tumor uptake.

Toxicity and Therapeutic Effect

All of the patients tolerated all the three cycles of therapy at the intended dose. Patients showed no acute or delayed major (grade 3 and 4) adverse reactions after the intravenous injection of ^{90}Y-DOTATOC up to a maximum dose of 2.2 GBq per cycle. Three patients (15%) manifested moderate gastrointestinal toxicity: grade 2 nausea (intake decreased but able to eat) and 1 patient reported grade 1 vomiting (1 episode in 24h). No other clinical manifestations of toxicity such as skin reaction, allergy or fever were observed in these patients. Figure 2 a, b represents a global toxicity evaluation after sixty treatments performed in twenty patients.

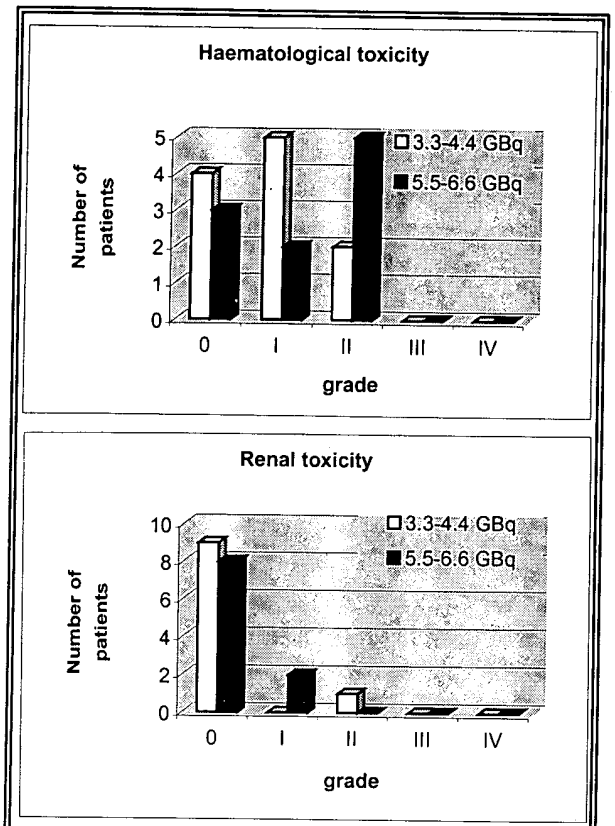

FIGURE 2: Haematological and renal toxicity according to WHO criteria. White bars refer to the first two groups of patients who received 3.3 and 4.4 GBq total dose, while the black bars refer to the 5.5 and 6.6 GBq groups, respectively.

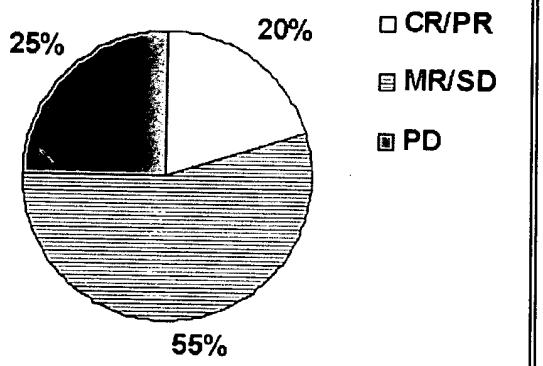

FIGURE 3: Summary of results obtained after the third cycle of therapy with ^{90}Y-DOTATOC. Follow-up period: 2-6 months (see text for details).

No major haematological toxicity (grade 3 and 4) was reported; in the range of 3.3 and 4.4 GBq, the majority of patients were in the grade 0-1 range. Only two patients presented with grade 2 haematological toxicity (haemoglobulin reduction) after 4.4 GBq, while the other five patients (black bar) with grade 2 toxicity had received higher activities. The majority of patients had no acute kidney toxicity, although two patients showed grade 1 and one had delayed grade 2 toxicity. This patient received a cumulative dose of 3.3 GBq. He was a 58 year old man affected by severe hypertension for six years. He had several liver metastases from a primary bronchous carcinoid. Although the serum creatinine was normal prior to therapy it is likely that even a relatively low dose of ~12 Gy resulted toxic to his kidneys damaged by hypertension too.

Figure 3 represents the overall therapeutic results for patients according to WHO evaluation. Five patients (25%) did not respond to therapy, eleven (55%) had minor or stable disease and four (20%) showed substantial tumor regression (CR/PR).

A CR example is illustrated in Fig 4. CT scan before therapy (a) revealed metastases in the liver parenchyma; CT scan (b) disappearance of all lesions after the third cycle of 1.1 GBq.

Chromogranin A level (c) dropped within normal range after the second cycle; a spot view of the abdomen showing ^{111}In- DOTATOC uptake in the liver lesions (d).

Fifty-five percent of the patients were classified as minor response or stable disease. An example is illustrated in Figure 5. Note the good match between In-111 imaging (a) and Y-90 bremsstrahlung imaging (b). An extremely high concentration of both tracers is evident in the liver lesions. Faint uptake was in the rest of the body including kidneys. As a result of therapy, the majority of small liver lesions

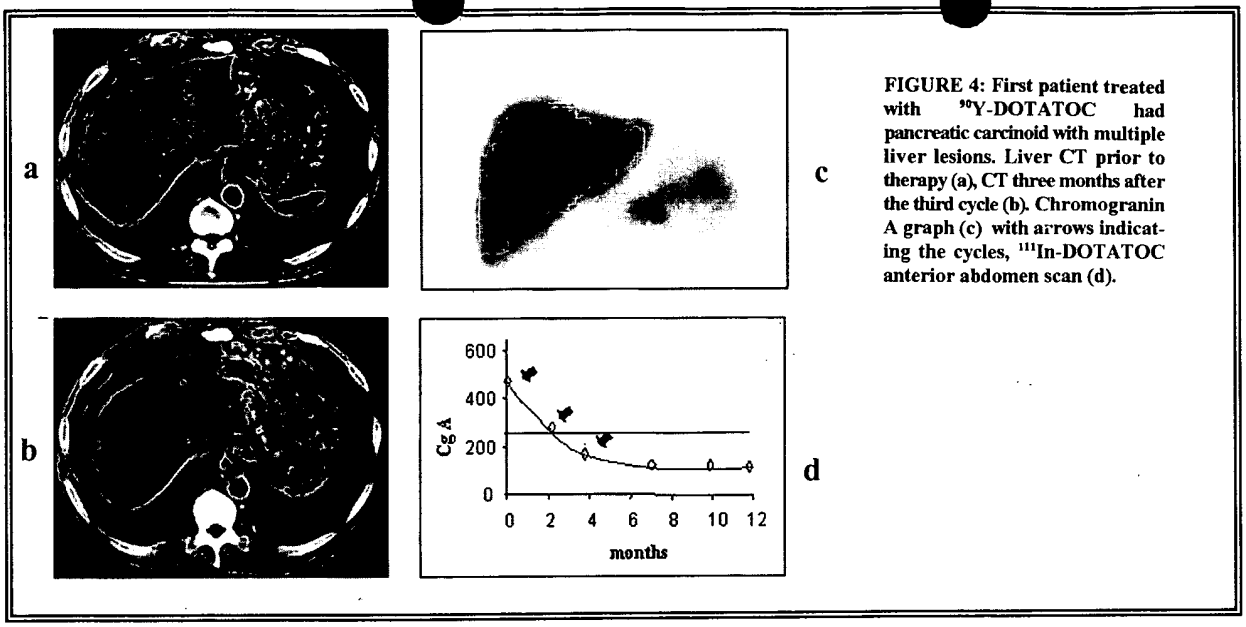

FIGURE 4: First patient treated with ^{90}Y-DOTATOC had pancreatic carcinoid with multiple liver lesions. Liver CT prior to therapy (a), CT three months after the third cycle (b). Chromogranin A graph (c) with arrows indicating the cycles, ^{111}In-DOTATOC anterior abdomen scan (d).

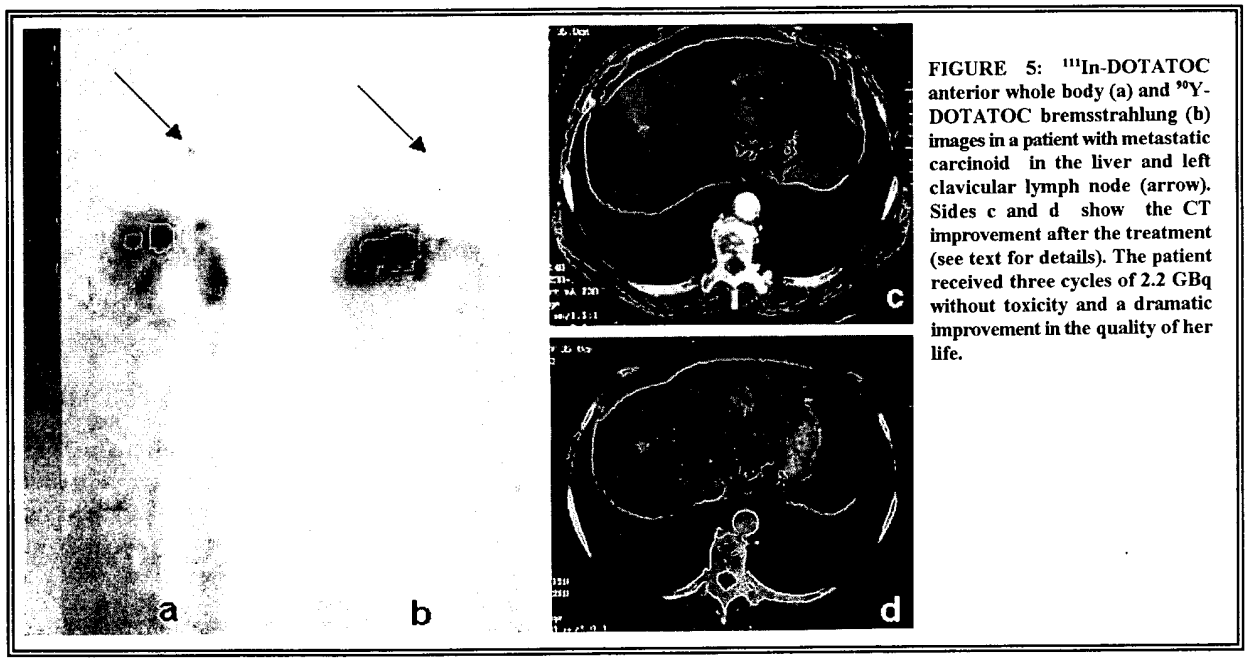

FIGURE 5: ^{111}In-DOTATOC anterior whole body (a) and ^{90}Y-DOTATOC bremsstrahlung (b) images in a patient with metastatic carcinoid in the liver and left clavicular lymph node (arrow). Sides c and d show the CT improvement after the treatment (see text for details). The patient received three cycles of 2.2 GBq without toxicity and a dramatic improvement in the quality of her life.

disappeared whereas the largest lesion in the VIII segment is unmodified although there are clear signs of central necrosis and lower enhancement (5c pre-therapy and 5d post-therapy). Since not all lesions were reduced more than 50%, this patient was classified as objective minor response according to the WHO criteria.

DISCUSSION

Although this is a preliminary report, conducted in a small number of patients, some points of discussion can be addressed. First of all, ^{90}Y-DOTATOC was repeatedly injected without allergic reactions at a dose as high as 60 μg for three times, as also observed by others.[22] The administration of a larger dose of DOTA conjugated somatostatin analogues may evoke an immunoresponse to DOTA as previously reported for DOTA-antibody complex.[23] Although in our opinion this is quite unlikely, we are currently investigating this aspect in the sera of our patients (study in progress).

Second, dosimetric calculations and clinical outcomes clearly showed that high doses of ^{90}Y-

DOTATOC can be injected without myelotoxicty. We have some concern about kidney and spleen radiation absorbed doses although no major toxic effects have been observed so far. Assuming the same biodistribution of $^{111}In/^{90}Y$-DOTATOC the predicted absorbed dose to these critical organs after the administration of 3.7 GBq of ^{90}Y-DOTATOC would be 12.2 ± 8.1 Gy for kidneys, 28.1 ± 23.3 Gy for spleen and only 0.1 ± 0.04 Gy for red marrow. Therefore, the kidneys, or the spleen, are likely to be the dose-limiting organs in patients treated with ^{90}Y-DOTATOC. So far, only one patient in the group receiving 3.3 GBq of ^{90}Y-DOTATOC developed a delayed grade 2 renal toxicity. This was probably due to baseline kidney function already impaired by a long history of hypertension even though the serum levels of creatinine were in the normal range before the therapy. Thus, a careful renal evaluation would be recommended and now, we prefer to assess the kidney function performing glomerular filtration rate and/or creatinine clearance before therapy. At present, some methods to reduce renal uptake have been explored based on the administration of cold octreotide[24] or of amino-acids.[11,25,26] Animal studies have shown a significant reduction of kidney uptake (50%-60%) after the intravenous administration of D-lysine without affecting the blood clearance.[11,25] The efficacy of these kidney protection methods to the current therapy trials is under investigation in our Institute.

The very high dose to the spleen (30-60 Gy / 3.7-7.4 GBq) could to produce splenic atrophy.[27] Spleen ablation can increase by about 10% the risk of septic shock over the normal population. However, the benefit in terms of tumor regression and the possibility of preventive antipneumococcal vaccination can justify this receptor mediated radionuclide therapy.

The absorbed dose to the red marrow seems to be negligible with this therapy. However, when therapy with higher doses (3.7-7.4 GBq) of ^{90}Y-DOTATOC is planned, free ^{90}Y in the radiopharmaceutical preparation must be carefully determined.

Finally, we observed objective responses in 20% of the patients and 55% had stable disease (no change or regression < 25%) lasting at least for three months with subjective and laboratory improvement (tumor markers).

The DOTATOC uptake observed in tumors, was high and stable and quite suitable for efficient receptor-mediated radiotherapy. Residence time in tumors showed variability (mean value: 0.5h; range: (0.05– 6.5h), giving absorbed doses to the tumor of 10.1 (1.4–31.0) mGy/MBq of ^{90}Y-DOTATOC. The variability could be related to factors such as tumor volume, interstitial pressure, viability etc. Moreover, variable receptor density on the tumors, after previous treatments, could affect the accumulation of tumor receptor binding radiotracers.

We have followed a horizontal protocol administering a sequence of equivalent doses to the same patient with intervals long enough to monitor sub-acute and late toxicity. It is still to be assessed whether positive tumor responses are expected after a single cycle of the highest dose injectable or after multiple low-dose treatments. Further studies are needed to determine the MTD and to evaluate the effect of number and interval between cycles versus single administration.

CONCLUSIONS

Overall, the results of this pilot therapeutic study confirmed the possibility of delivering high radiation doses to the tumor using ^{90}Y-DOTATOC; objective therapeutic responses were observed. Promising methods of reducing uptake in some organs (specifically kidneys) are under study and will probably allow further increasing the administered ^{90}Y-DOTATOC dose.

ACKNOWLEDGMENTS

This work was supported by grants from the Italian Association for Cancer Research (AIRC) and the Swiss National Science Foundation.

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Volume 14, Number 6, 1999
ISSN: 1084-9785

Cancer Biotherapy & Radiopharmaceuticals

A Journal of New Cancer Treatment Modalities

Editors
Robert K. Oldham, M.D.
Gerald L. DeNardo, M.D.

Mary Ann Liebert, Inc. publishers

[^{177}Lu-DOTA^0,Tyr^3]octreotate: comparison with [^{111}In-DTPA^0]octreotide in patients

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Received 26 February and in revised form 24 April 2001 / Published online: 4 July 2001

Abstract. The somatostatin analogue [DOTA^0,Tyr^3]octreotate has a nine-fold higher affinity for the somatostatin receptor subtype 2 as compared with [DOTA^0, Tyr^3]octreotide. Also, labelled with the beta- and gamma-emitting radionuclide lutetium-177, this compound has been shown to have a very favourable impact on tumour regression and animal survival in a rat model. Because of these reported advantages over the analogues currently used for somatostatin receptor-mediated radiotherapy, we decided to compare [^{177}Lu-DOTA^0,Tyr^3]octreotate (^{177}Lu-octreotate) with [^{111}In-DTPA^0]octreotide (^{111}In-octreotide) in six patients with somatostatin receptor-positive tumours. Plasma radioactivity after ^{177}Lu-octreotate expressed as a percentage of the injected dose was comparable with that after ^{111}In-octreotide. Urinary excretion of radioactivity was significantly lower than after ^{111}In-octreotide, averaging 64% after 24 h. The uptake after 24 h, expressed as a percentage of the injected dose of ^{177}Lu-octreotate, was comparable to that after ^{111}In-octreotide for kidneys, spleen and liver, but was three- to fourfold higher for four of five tumours. The spleen and kidneys received the highest absorbed doses. The doses to the kidneys were reduced by a mean of 47% after co-infusion of amino acids. It is concluded that in comparison with the radionuclide-coupled somatostatin analogues that are currently available for somatostatin receptor-mediated radiotherapy, ^{177}Lu-octreotate potentially represents an important improvement. Higher absorbed doses can be achieved to most tumours, with about equal doses to potentially dose-limiting organs; furthermore, the lower tissue penetration range of ^{177}Lu as compared with ^{90}Y may be especially important for small tumours.

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Keywords: Somatostatin – Somatostatin receptor imaging – Octreotate – Peptide receptor radiotherapy

Eur J Nucl Med (2001) 28:1319–1325
DOI 10.1007/s002590100574

Introduction

Somatostatin receptor imaging with [^{111}In-DTPA^0]octreotide (Octreoscan) is nowadays recognised to be an important, if not the primary imaging technique for the localisation and staging of neuroendocrine tumours.

In patients with progressive, metastasised neuroendocrine tumours, radionuclide therapy with high doses of [^{111}In-DTPA^0]octreotide is performed with encouraging results [1, 2, 3, 4]. However, ^{111}In-coupled peptides are not ideal for peptide receptor radiotherapy (PRRT) because of the small particle range and the resultant short tissue penetration. Therefore, another radiolabelled somatostatin analogue, [^{90}Y-DOTA^0,Tyr^3]octreotide, was developed. A preliminary study by Otte et al. [5] showed favourable results of [^{90}Y-DOTA^0,Tyr^3]octreotide treatment in five patients with neuroendocrine tumours. Also, a recent analysis of the results of this treatment in a multicentre trial in 22 end-stage patients with progressive disease showed a partial tumour response in two, a minor response in three and stable disease in ten [6]. Paganelli et al. [7] have also reported favourable preliminary results regarding tumour growth with this ^{90}Y-labelled compound.

Recently, it was reported that compared with [DTPA^0, Tyr^3]octreotide, [DTPA^0,Tyr^3]octreotate (in which the C-terminal threoninol is replaced with threonine) showed improved binding to somatostatin receptor-positive tissues in animal experiments [8]. Also, its DOTA-coupled counterpart, [DOTA^0,Tyr^3]octreotate, labelled with the

beta- and gamma-emitting radionuclide lutetium-177, was reported to have a very successful impact on tumour regression and animal survival in a rat model [9]. Reubi et al. [10] reported a ninefold increase in affinity for the somatostatin receptor subtype 2 for [DOTA^0,Tyr^3]octreotate as compared with [DOTA^0,Tyr^3]octreotide, and a six- to sevenfold increase in affinity for their yttrium-loaded counterparts.

Because of these reported advantages over both somatostatin analogues currently used for PRRT, we decided to study [DOTA^0,Tyr^3]octreotate in patients with somatostatin receptor-positive tumours. It was complexed with ^{177}Lu because this radionuclide, apart from intermediate beta energy, also emits gammas suitable for scintigraphy and subsequent dosimetry.

Materials and methods

Patients

[^{177}Lu-DOTA^0,Tyr^3]octreotate (^{177}Lu-octreotate) was administered in six patients (four women and two men, aged 15–76 years). In five of them, somatostatin receptor imaging with [^{111}In-DTPA^0]octreotide (^{111}In-octreotide), performed during the 3 months preceding ^{177}Lu-octreotate scintigraphy, was available. None of the patients used somatostatin analogues.

One patient had medullary thyroid carcinoma (MTC), one had non-Hodgkin lymphoma (NHL), one had a gastroenteropancreatic (GEP) tumour, one had aesthesioneuroblastoma, one had a remnant of a Hürthle cell carcinoma of the thyroid, and one had papillary thyroid carcinoma.

All patients gave written informed consent to participation in the study, which was approved by the medical ethical committee of the hospital.

Methods

[DOTA^0,Tyr^3]Octreotate was obtained from Mallinckrodt (St Louis, Mo., USA). Kits were prepared consisting of 120 µg [DOTA^0,Tyr^3]octreotate, 37.8 mg sodium ascorbate and 7.5 mg gentisic acid in 300 µl 0.05 *M* HCl. Kits were stored at –20°C until use. $^{177}LuCl_3$ was obtained from Missouri University Research Reactor (MURR; University of Missouri, Mo., USA). $^{177}LuCl_3$ was diluted in 0.05 *M* HCl to a concentration of 11.1 GBq/ml, and 2,220 MBq $^{177}LuCl_3$ was added to each kit. The mixture was heated for 30 min at 80°C. The labeling yield was checked using instant thin-layer chromatography (ITLC-SG, Gelman, Ann Arbor, Mich., USA) with 0.1 *M* Na citrate, pH 5.0, as solvent. The labelled peptide migrated from the origin till Rf=0.67, while the free radionuclide migrated with the solvent front (Rf=1).

The radiochemical purity was determined by high-performance liquid chromatography (HPLC) according to the following procedure. Column: Symmetry C_{18} 4.6×250 mm, 5 µm (Waters, Milford, Mass., USA). Flow: 1 ml/min. Solvent A: methanol; solvent B: 0.06 *M* sodium acetate pH 5.5. From *t*=0 to 6.5 min 100% B; from *t*=6.5 to 7.0 min from 100% B to 50% B; from *t*=7.0 to 27 min from 50% B to 40% B; from *t*=27 min to 27.2 min from 40% B to 100% A; from *t*=27.2 min to 32 min: 100% A.

The labeling yield always exceeded 98% and the radiochemical purity was higher than 88%. The injected dose was 1,850 MBq (range 1,847–1,874 MBq); the injected mass of [DOTA^0,Tyr^3]octreotate was 90–100 µg.

^{111}In-octreotide was prepared using the Octreoscan kit from Mallinckrodt Medical (Petten, the Netherlands). The injected dose was about 220 MBq, coupled to 8–9 µg [DTPA^0]octreotide.

Imaging

^{177}Lu-octreotate. The infusion volume was 80 ml and the infusion speed was 10 min. The infusion line by which the radiopharmaceutical was administered was thereafter rinsed with about 100 ml saline. Dynamic images of the upper abdomen were obtained from the time of injection up to 20 min p.i. Planar spot images of the upper abdomen and chest in five patients, and of the upper abdomen and the head and neck in the sixth patient, were obtained with a dual-head camera (Picker Prism 2000) 4 h and 1, 3, 10 and 17 days p.i. Counts from both gamma peaks (208 and 113 keV) were collected in separate windows (width 20%). The acquisition time was 15 min/view. For dosimetry, a standard with a known aliquot of the injected dose was also counted.

^{111}In-octreotide. The windows were centered over both ^{111}In photon peaks (245 and 172 keV) with a window width of 20%. Fifteen-minute spot images were obtained 24 h p.i.

Co-infusion of amino acids

In five patients the administration of the same amount and dose of ^{177}Lu-octreotate was repeated 6–9 weeks later. An infusion of amino acids (lysine 2.5%, arginine 2.5% in 1 l 0.9% NaCl; 250 ml/h) was started 30 min before the administration of the radiopharmaceutical and lasted up to 3.5 h afterwards. Via a second pump system the radiopharmaceutical was co-administered.

Measurement of radioactivity in blood and urine

Blood samples were drawn 10, 20, 40, 60 and 90 min and 2, 5 and 24 h after injection. Urine was collected at two 3-h intervals and thereafter up to 24 h after injection.

Radioactivity in blood and urine was measured with a COBRA-Packard auto-gamma counting system (Packard, Meriden, Conn., USA).

The chemical status of the radionuclide in blood and urine was analysed as a function of time by HPLC techniques (see above).

In vivo measurements

The uptake in organs and tumours was calculated as described previously [11]. Dosimetric calculations were performed using the MIRDOSE package, version 3.0.

Statistics

Analysis of variance (ANOVA) and paired *t* tests were used. *P* values <0.05 were considered significant.

Results

No side-effects or changes in ECG pattern or pulse rate were observed in any patient during the 10-min infusion

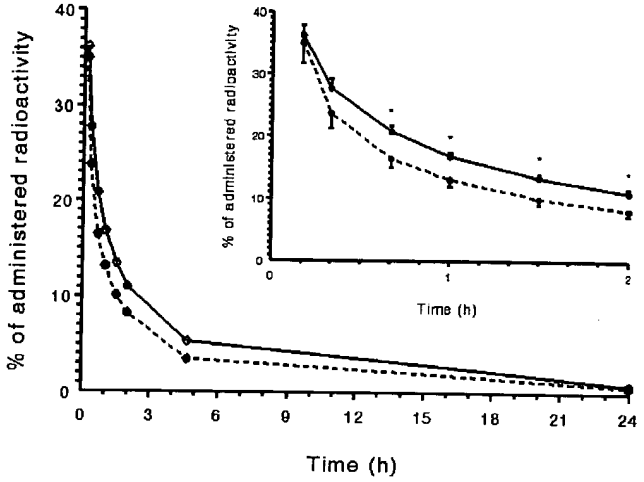

Fig. 1. Mean (±SEM) plasma radioactivity expressed as percentage of the injected dose in six patients after ^{177}Lu-octreotate (*closed dots, stippled line*), compared with that in four other patients after ^{111}In-octreotide from a previous study [12] (*open dots, solid line*). *P<0.05 vs other radiopharmaceutical at the same time point

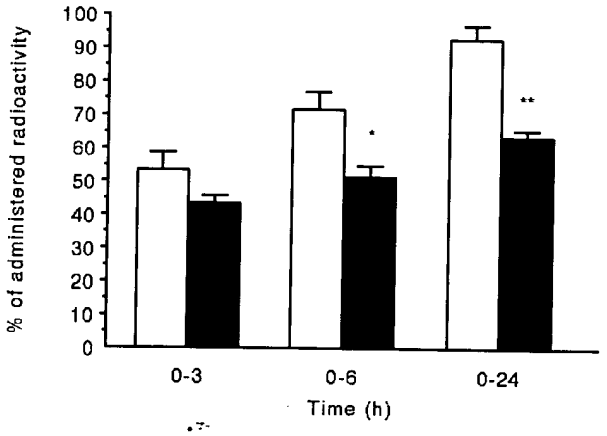

Fig. 2. Cumulative radioactivity excreted in the urine, expressed as mean (±SEM) percentage of the injected dose in four patients after ^{177}Lu-octreotate (*closed bars*), compared with that in six other patients after ^{111}In-octreotide from a previous study [12] (*open bars*). *P<0.05 and **P<0.01 vs other radiopharmaceutical during the same interval

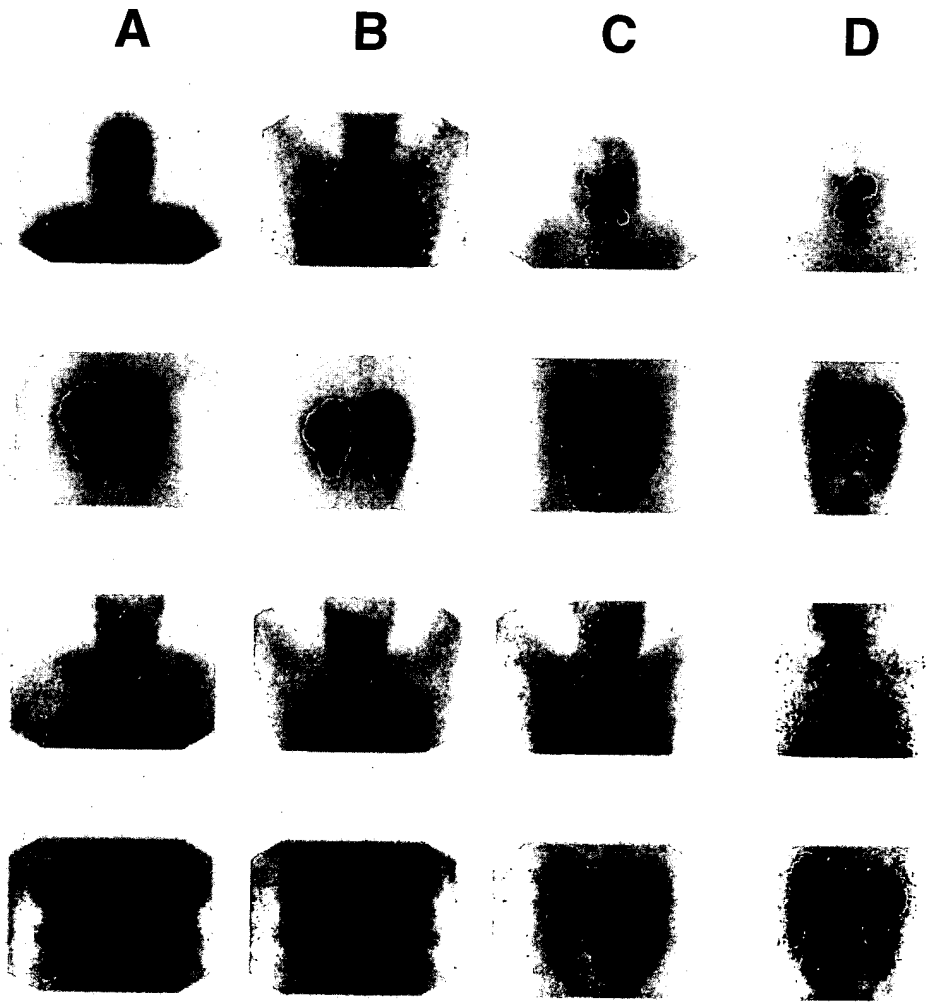

Fig. 3. Images comparing ^{177}Lu-octreotate and ^{111}In-octreotide, 24 h p.i. *Columns A and C:* ^{177}Lu-octreotate; *columns B and D:* ^{111}In-octreotide. The *first row* shows corresponding images of tumour sites in a lymphoma patient (left two images) and a patient with an aesthesioneuroblastoma of the eye with a neck metastasis (right two images); *second row:* posterior (left two images) and anterior abdominal images in the same patients. The *third row* shows corresponding images of tumours in a patient with residual Hürthle cell carcinoma (left two images) and a patient with papillary thyroid carcinoma (right two images); *fourth row:* anterior abdominal images in the same patients. Note the similar biodistribution and the clearer visualisation of the tumour sites, except in the patient with papillary thyroid carcinoma

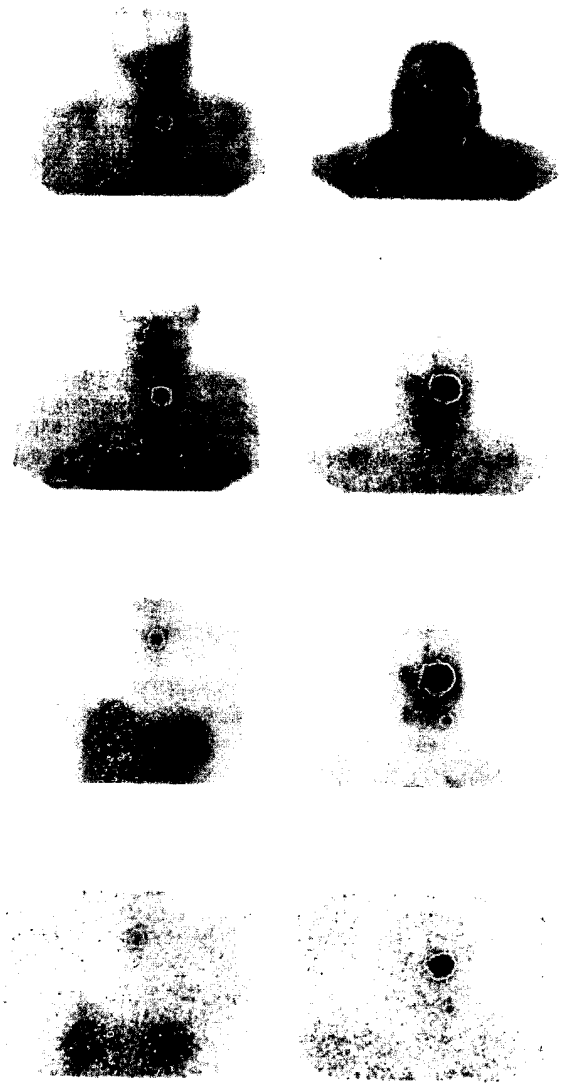

Fig. 4. Images after 4 h and 1, 3 and 17 days (*top row to lower row*) in patients with Hürthle cell carcinoma (*left column*) and aesthesioneuroblastoma (*right column*). Note the retention of radioactivity in the tumour sites

of ^{177}Lu-octreotate or up to 20 min thereafter. The distribution pattern of ^{177}Lu-octreotate was comparable to that of ^{111}In-octreotide, with rapid visualisation of the kidneys directly after injection, and with visualisation of the liver, spleen, kidneys and, in some patients, the pituitary, thyroid and tumours 4 h p.i.

Plasma radioactivity after ^{177}Lu-octreotate expressed as a percentage of the injected dose was slightly, but significantly lower compared with ^{111}In-octreotide measurements from a previous study [12]. After 24 h, however, they were comparable (Fig. 1).

HPLC analysis of plasma, taken at 1 h p.i. in two patients, demonstrated the same pattern as the original injection fluid (data not shown).

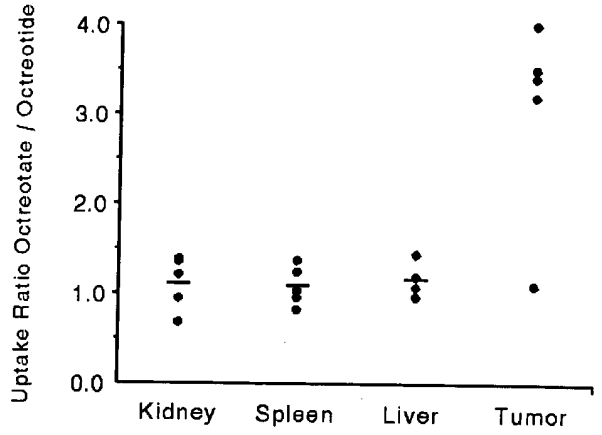

Fig. 5. Ratios of ^{177}Lu-octreotate to ^{111}In-octreotide uptake in organs and tumour sites, with uptake expressed as a percentage of the administered dose. Means are indicated. There is comparable organ uptake and higher tumour uptake after ^{177}Lu-octreotate in most tumours

Table 1. Patient organ doses in cGy (rad)/3,700 MBq (100 mCi)

Patient	Kidneys		Liver	Spleen	Bone marrow
	Without AA	With AA			
1	825	403	90	803	26
2	533	–	76	1,010	29
3	692	282	112	770	27
4	359	252	44	662	27
5	648	366	75	740	20
Mean	611	326	79	797	26

With AA: Kidney dose after amino acid co-infusion

Urinary excretion of radioactivity in the first 24 h after the injection of ^{177}Lu-octreotate is shown in Fig. 2. In comparison with ^{111}In-octreotide, the urinary excretion was significantly lower after ^{177}Lu-octreotate, averaging 64% after 24 h. Peptide-bound radioactivity in urine collected after 1 h in one patient showed the same pattern as the original injection fluid (data not shown).

The scans obtained 24 h p.i. showed the same biodistribution for ^{177}Lu-octreotate and ^{111}In-octreotide, with comparable uptake in the liver, spleen and kidneys (Fig. 3). Also, variable radioactivity was seen in the bowel and urinary bladder. The uptake in the tumours seemed higher after ^{177}Lu-octreotate, except in the patient who had papillary thyroid carcinoma (Fig. 3). At later time points, there was retention of the radioactivity in the tumours, even 17 days p.i. (Fig. 4). The calculated, background-corrected, uptake 24 h after ^{177}Lu-octreotate expressed as a percentage of the injected dose was comparable to that after ^{111}In-octreotide for kidneys, spleen and liver, but was three- to fourfold higher for four of the five tumours (Fig. 5). In the patient with papillary thyroid carcinoma, this uptake was about the same after both radiopharmaceuticals.

Table 2. Dose estimates for ^{177}Lu-octreotate, ^{111}In-octreotide and ^{90}Y-DOTA-octreotide

Target organ	Absorbed dose [cGy (rad)/3,700 MBq (100 mCi)][a]							
	^{177}Lu-octreotate	^{111}In-octreotide			^{90}Y-DOTA-octreotide			
		(1)	(2)	(3)	(4)	(5)	(6)	(7)
Kidneys[b]	610 (325)	170	190	340	2,240	1,220	1,040 (780)	(788)
Liver	80	30	25	90	100	260	120	–
Spleen	800	120	130	320	1,980	2,820	–	–
Bone marrow	26	7	11	23	–	11	26	–
Maximum cumulative dose (GBq)[c]	26.4	50.0	44.8	25.0			10.9	10.8
Maximum cumulative dose (mCi)[c]	710	1,350	1,210	680			290	290

[a] Dosimetry data reported by: (1) Krenning et al. [13]; (2) Stabin et al. [14]; (3) McCarthy et al. [15]; (4) Kwekkeboom et al. [12] (dosimetry based on [^{111}In-DOTA^0,Tyr^3]octreotide); (5) Cremonesi et al. [16] (dosimetry based on [^{111}In-DOTA^0,Tyr^3]octreotide); (6) Rosch et al. [17] (dosimetry based on [^{86}Y-DOTA^0, Tyr^3]octreotide PET studies in primates); (7) Barone et al. [18] (dosimetry based on [^{86}Y-DOTA^0,Tyr^3]octreotide PET studies in patients)

[b] Kidney doses after amino acid co-infusion are shown within parentheses

[c] Mean maximum cumulative dose based on the maximum dose to the kidneys of 23 Gy. Values for ^{177}Lu-octreotate and ^{90}Y-DOTA-octreotide are with amino acid co-infusion

Table 3. Theoretical maximum delivered tumour doses for three different radiolabelled somatostatin analogues

	Absorbed dose (Gy)		
	^{111}In-octreotide	^{90}Y-DOTA-octreotide	^{177}Lu-octreotate
Tumour 1 g	344	563	1,001
Tumour 10 g	36	69	102
Tumour uptake	0.1%	0.2%	0.4%
Maximum cumulative dose [mCi (GBq)]	1,350 (50.0)	290 (10.9)	710 (26.4)

Maximum cumulative doses are derived from Table 2. The calculations take into account the fact that the tumour uptake of [^{90}Y-DOTA,Tyr^3]octreotide is about two times higher [18], and that of ^{177}Lu-octreotate about four times higher, (present study) than the tumour uptake of ^{111}In-octreotide

Dosimetry data are listed in Table 1. The highest absorbed doses were to spleen and kidneys. In five patients, scintigraphy was repeated several weeks later, after co-infusion of amino acids (lysine 2.5%, arginine 2.5%; 250 ml/h for 4 h, starting 30 min before the administration of ^{177}Lu-octreotate). Calculated doses to the liver, spleen, bone marrow and tumours were about the same, whereas the doses to the kidneys were reduced by a mean of 47% (range 34%–59%) (Table 1).

The dose estimates after ^{177}Lu-octreotate are compared with those after ^{111}In-octreotide and [^{90}Y-DOTA,Tyr^3]octreotide in Table 2. Compared with [^{90}Y-DOTA,Tyr^3]octreotide, the dose to the spleen and kidneys was lower after ^{177}Lu-octreotate, whereas the dose to the bone marrow was comparable or higher, depending on the model that was used. Theoretical maximum tumour doses for ^{111}In-octreotide, [^{90}Y-DOTA,Tyr^3]octreotide, and ^{177}Lu-octreotate, based on a maximum kidney dose of 23 Gy, are listed in Table 3. The highest tumour doses in this model are achieved with ^{177}Lu-octreotate, especially in smaller tumours.

Discussion

The somatostatin analogue Tyr^3-octreotate, whether chelated with DTPA or DOTA, has been demonstrated to have a higher affinity than Tyr^3-octreotide for the most frequently encountered somatostatin receptor, subtype 2, both in vitro and in vivo in animal experiments [8, 9, 10]. Because the total administered therapeutic dose with radiolabelled somatostatin analogues is determined by organ dose limits, any newly developed analogues that show either a lower uptake in the dose-limiting organs (kidneys and/or bone marrow) or a higher uptake in the tumour targets, may improve such therapy. For this reason, we compared the distribution pattern and dosimetry of ^{177}Lu-octreotate with those of ^{111}In-octreotide in patients. We found the same biodistribution for the analogues on scintigrams 24 h p.i., with comparable percentage uptake in the liver, spleen and kidneys. The tumour uptake, however, was three- to fourfold higher in four of the five studied patients, implying that potentially higher doses to the tumour can be achieved with about

equal dose-limiting organ doses. The comparability of the percentage uptake in the liver and kidneys for the two analogues is most likely due to the fact that the uptake in these organs is for the most part not receptor mediated and is accounted for by excretion of the radiopharmaceutical. Our finding that the uptake in the spleen was comparable for the two analogues may indicate that a considerable part of this uptake is due to binding to a somatostatin receptor subtype other than subtype 2. Somatostatin receptor subtype 2 has been demonstrated in the red pulp of the spleen by autoradiography [19]; however, with reverse transcriptase polymerase chain reaction (RT-PCR) techniques, mRNA for receptor subtype 3 has also been demonstrated, located in the white pulp and with a much lower expression than for subtype 2 [20]. It is therefore puzzling why the scintigraphic uptake in the spleen after ^{177}Lu-octreotate is not much higher than after ^{111}In-octreotide. The presence of other somatostatin receptor subtypes may explain why, in our patient with papillary thyroid carcinoma, uptake was not higher with ^{177}Lu-octreotate than with ^{111}In-octreotide. This is in agreement with an in vitro study using RT-PCR on human thyroid carcinoma cell lines, which demonstrated a predominance of mRNA for somatostatin receptor subtypes 3 and 5, but only very low amounts of mRNA for subtype 2 [21].

We found a comparable, but slightly faster plasma disappearance for ^{177}Lu-octreotate than for ^{111}In-octreotide. More importantly, the cumulative urinary excretion after 24 h was significantly lower for ^{177}Lu-octreotate than for ^{111}In-octreotide. In another study [12], we found that the cumulative urinary excretion of [^{111}In-DOTA^0,Tyr^3]octreotide was also significantly lower than that of [^{111}In-DTPA^0]octreotide. Because this analogue has the tyrosine insertion in common with [^{177}Lu-DOTA^0,Tyr^3]octreotate, this may be the cause of the lower urinary excretion. This lower urinary excretion of ^{177}Lu-octreotate results in a significantly higher absorbed bone marrow dose, because this dose is determined by the whole-body retention (i.e. injected minus excreted radioactivity minus activity in major target organs). Because of this, both the radiation dose to the kidneys and that to the bone marrow may be dose limiting in patient therapy with ^{177}Lu-octreotate. It has previously been demonstrated that the percentage uptake by and the radiation dose to the kidneys from ^{111}In-octreotide can be lowered by the infusion of amino acids, both in animals and in patients [22, 23]. In our group of patients, we found that this is also true for ^{177}Lu-octreotate. This finding is important because applying a dose limit to the kidneys of 23 Gy, as is also applied for external beam radiation therapy, 14.0 GBq (380 mCi) would be the mean cumulative dose limit, whereas with the reduction due to amino acid infusion this limit would be 26.4 GBq (710 mCi). Barone et al. [18] compared the uptake after ^{111}In-octreotide and after [^{86}Y-DOTA^0,Tyr^3]octreotide in the tumours and kidneys of five patients. They found that the percentage uptake in tumours was about two times higher after [^{86}Y-DOTA^0,Tyr^3]octreotide, whereas it was about 1.4 times higher in the kidneys. Applying a dose limit of 23 Gy to the kidneys and accounting for amino acid co-infusion, the maximum cumulative dose of [^{90}Y-DOTA^0,Tyr^3]octreotide would be 10.8 GBq (290 mCi) (Table 2). Using ^{177}Lu-octreotate, the mean maximum cumulative dose that can be administered is 26.4 GBq (710 mCi) (Table 2). Apart from the more than double mean maximum cumulative dose that can be administered using ^{177}Lu-octreotate instead of [^{90}Y-DOTA^0,Tyr^3]octreotide, it should be considered that in this study we found a three- to fourfold higher tumour uptake with ^{177}Lu-octreotate than with ^{111}In-octreotide, whereas with [^{86}Y-DOTA^0,Tyr^3]octreotide the tumour uptake was reported to be about twofold that of ^{111}In-octreotide [18]. In a model calculation, based on the maximum cumulative dose that can be given, the tumour doses that can be achieved with ^{177}Lu-octreotate were higher than those for ^{111}In-octreotide or [^{90}Y-DOTA^0,Tyr^3]octreotide.

There are four reasons why we used ^{177}Lu and not ^{90}Y as the radionuclide to label [DOTA^0,Tyr^3]octreotate:

1. ^{177}Lu-octreotate has been reported to have a very favourable impact on tumour regression in a rat model [9].
2. Reubi et al. [10] reported comparable affinities in the low nanomolar range for non-radioactive In and Y complexed [DOTA^0,Tyr^3]octreotate, implying that the modification in the peptide, and not the change in the metal, is primarily responsible for the improved affinity.
3. With the ^{177}Lu-labelled analogue it is possible to perform dosimetry and therapy with the same compound while no PET scans with short-lived radionuclides are needed.
4. The tissue penetration range of ^{177}Lu (maximum range ≈2 mm) is more favourable than that of ^{90}Y (maximum range ≈12 mm), especially for smaller tumours from which much of the radiation dose of ^{90}Y will be lost to the surrounding tissues.

Because of the advantages that both the modified somatostatin analogue and the different radionuclide offer, we think that ^{177}Lu-octreotate represents an improvement in somatostatin receptor-mediated radiotherapy. Indeed, we have already observed improvements in complaints, decreases in serum tumour markers and CT-assessed tumour shrinkage in patients who are being treated with this new compound, although none of these patients has yet received the maximum cumulative dose.

In conclusion: In comparison with the radionuclide-coupled somatostatin analogues that are currently available for somatostatin receptor-mediated radiotherapy, ^{177}Lu-octreotate potentially represents an important improvement because of the higher absorbed doses that can be achieved to most tumours and because of the more fa-

vourable tissue penetration range, which may be especially important for small tumours.

Acknowledgements. The authors want to thank all the co-workers of the technical staff of the Department of Nuclear Medicine for their expert cooperation.

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European Journal of Nuclear Medicine

28/9
2001

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US 096767830IP1

Creation date: 12-17-2003
Indexing Officer: TNGUYEN64 - TUAN NGUYEN
Team: OIPEBackFileIndexing
Dossier: 09676783

Legal Date: 11-10-2003

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